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An Investigation of the Sulfonic Acids Derived from Xylose and Arabinose

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AN INVESTIGATION OF THE SULFONIC ACIDS DERIVED FROM XYLOSE AND ARABINOSE

A thesis submitted by

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INTRODUCTION

Acid sulfite pulping of wood is one of the more important sources of fiber for use in the pulp and paper industry (3,180,000 tons produced in 1957)(1). In addition, the production of wood pulp by the neutral sulfite process is increasing steadily (1,330,000 tons produced in 1955, 1,490,000 tons in 1957)(1). The spent pulping liquors produced by these processes constitute a vast potential source of numerous products; however, before these liquors can be used economically, more data concerning their nature and composition are needed.

The sulfonation of the lignin component of wood has been extensively investigated, but the reactions of the carbohydrate fraction have not been thoroughly studied with regard to possible transformation into sulfonated derivatives. Although several workers (2-5) have found evidence that sulfonated carbohydrate materials may be formed during acid sulfite pulping of wood, the available information is limited, and proof of the existence of these substances is lacking. Because of the very complex nature of spent sulfite liquors with attendant problems of isolation of pure materials, several workers have studied the sulfonation of simple carbohydrates with particular attention to the products derived from the simple sugars (6-8, 9-12). Such work is warranted as a first step in gaining knowledge concerning the chemical and physical properties of this class of compounds. It was with the intent of obtaining additional information concerning the nature of these materials that the present study was undertaken.

HISTORICAL REVIEW

The first evidence that sulfonated carbohydrates are formed during acid sulfite pulping of wood was presented by Erdtman (2). The acidic components in a sulfite pulping liquor were divided into four broad fractions which were classified as follows:

1. The barium salts of the high molecular weight acids which were precipitated by 4-4'-bisdimethylaminodiphenylmethane sulfate.
2. The barium salts of lower molecular weight acids which were isolated by an incomplete precipitation with brucine.
3. The barium salts of lower molecular weight acids which were precipitated by the addition of more brucine.
4. The barium salts of low molecular weight acids which were not precipitated by the above reagents, but which were obtained by precipitation of the barium salts in alcohol.

Fractions 1 and 2 were found to consist of lignosulfonic acids, but Fractions 3 and 4 were contaminated with materials which probably were "carbohydrate sulfonic acids or joined with such acids with the formation of glucosides" (2).

Subsequently, other investigations (3-5) have supported Erdtman's hypothesis that sulfonated carbohydrates are produced during acid sulfite pulping of wood. However, these acids have not been isolated in a pure form so that they have not been thoroughly investigated. In general, low molecular weight acidic fractions which contained only small amounts of

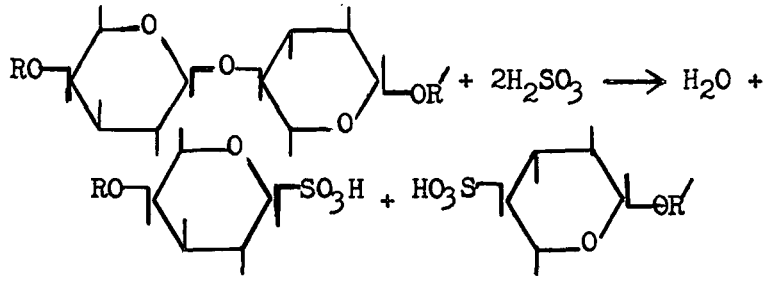
lignosulfonic acids as shown by low methoxyl contents but which contained sulfur were studied. The sulfur was found to be in a relatively stable form toward both acids and alkalies so that it was not present as a carbonyl-bisulfite complex. Very little else is known about these sulfur-bearing materials.

In attempting to obtain pure products, holocelluloses containing only small amounts of lignin were subjected to the action of hot acid sulfite liquors (7, 9). Unfortunately, the products were still contaminated, mainly with aldonic acids. To quote Adler (7), "a certain amount of a carbohydrate derivative, poor in sulfur, is formed in addition to the aldonic acids, and this derivative is probably a sulfonic acid." The effect of acid and neutral sulfite solutions on lactose and cellobiose was studied, but no sulfur-bearing products were isolated (5).

Glucose was converted into the corresponding aldonic acid in acid sulfite liquors (6,7), but fractose yielded a sulfonic acid of undetermined structure under the same conditions (6, 10). A sulfonated product, tentatively identified as "levoglucosan-6-sulfonic acid", was prepared from glucose by the action of a bisulfite solution (11, 12). Hägglund and co-workers (6) and Adler (7) have studied the sulfonic acids derived from glucose upon treatment with a hot, nearly neutral (pH 6) sodium sulfite-bisulfite solution. One acid, which analyzed as $C_6H_{10}O_8S$ and which was isolated via its water-insoluble phenylhydrazone, contained an aldehyde, a carboxyl, a sulfonic acid, and a terminal glycol group. Another glucosulfonic acid, which analyzed as $C_6H_{12}O_8S$, was also isolated. This acid

contained a carboxyl, a sulfonic acid, and a terminal glycol group. The structure of these acids has not been fully established.

Since glucose did not form a sulfonic acid when treated with acid sulfite liquors, Hägglund and co-workers (6) concluded that the free monomeric aldose sugars cannot form sulfonated derivatives during acid sulfite pulping of wood. Adler (7) suggested that the sulfonated carbohydrates may be formed in a combination sulfonation-hydrolysis reaction as shown in Equation (1). This reaction, termed



(1)

"sulfitolysis" (7), is similar to the "phosphorolysis" reaction which has been noted in biological degradation of several polysaccharides. Upon further hydrolysis, monomeric sulfonic acids would be produced. The relationship between such acids as may be produced in acid sulfite pulping of wood and those prepared by treatment of the simple aldoses with neutral sulfite liquors is not known.

An extensive study of the sulfonic acids derived from xylose by treatment with a hot, nearly neutral (pH 6.5) solution of sodium sulfite-bisulfite has been conducted by Yllner (8). The acids were prepared by heating xylose in an aqueous solution of sodium sulfite (2.34 moles per mole xylose) and sodium pyrosulfite (2.34 moles as bisulfite per mole xylose) in a sealed glass tube at 135°C. for one hour. After cooling the light

yellow liquor was passed through a column of Amberlite IR-120 cation-exchange resin in the acid form to remove sodium ions. Sulfur dioxide was then removed by passing nitrogen through the resulting acid effluent. Saturated barium hydroxide solution was added until the solution was neutral (pH 7.5). The resulting barium sulfite and sulfate were removed by filtration, and the filtrate was concentrated under reduced pressure to a small volume.

The crude barium xylo-sulfonates were separated into four fractions by alcohol precipitation. The barium salts were optically inactive and contained barium and sulfur in a ratio of 0.93 - 0.97. This ratio indicated that the material was a sulfocarboxylic acid salt, and calculated as such the yield of crude acids was about 50% based on the initial xylose.

Chromatography of the crude acids as the ammonium salts in ethanol-water (4:1) separated fractions 1, 2, and 3 into one major spot (R_f = 0.20) and several minor spots. Fraction 4 separated into five spots with R_f values of 0.20, 0.26, 0.30, 0.36 and 0.40.

Colored impurities were removed from the crude barium salts by means of animal charcoal-celite mixtures. The resulting product was very nearly chromatographically homogeneous when studied using the technique described above. The barium salts, which were amorphous and optically inactive, analyzed as $C_5H_8O_7SBa$.

The brucine salts of the charcoal-purified acids were prepared, and

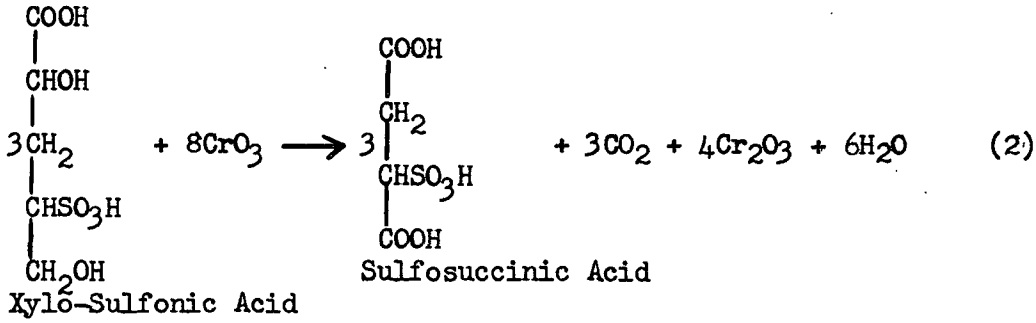
two fractions, differing in crystalline form, were isolated. One fraction, which had rod-shaped crystals, was isolated in approximately 50% yield according to Yllner (8). In addition, he isolated a second fraction which had needle-shaped crystals in a yield of about 25% on the basis of the initial charcoal-purified acids. This second fraction of the brucine salt was not investigated further.

The first modification of the brucine salt was converted into the barium salt[1] which crystallized from water-ethanol mixtures. The salt analyzed as $C_5H_8O_7SBa.H_2O$ and was optically inactive. The free acid was stable toward periodate, indicating the absence of a 1,2-glycol.

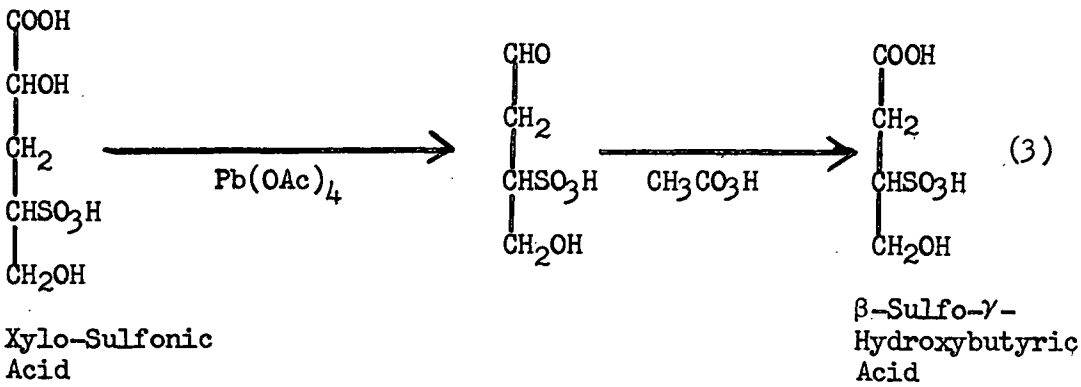
From the barium-to-sulfur ratio, the pure xylo-acid contained a sulfonic and a carboxyl group. Since the acid did not possess any reducing properties, Yllner assigned the remaining oxygen to two alcohol groups. Chromic acid oxidation indicated that one alcohol group was primary. The consumption of oxidant was approximately equal to that theoretically required to produce sulfosuccinic acid according to the hypothetical reaction shown in Equation (2) (2.67 mole CrO_3 or 4 atoms of oxygen per mole of xylo-sulfonic acid). No attempt to isolate the sulfosuccinic acid was reported.

Although the pure xylo-sulfonic acid was stable toward periodate, it was oxidized by lead tetraacetate to yield one mole of carbon dioxide per mole of xylo-sulfonic acid. The resulting sulfoaldehyde was not

[1] This material isolated by Yllner will be termed the "pure xylo-sulfonic acid" in this report.



isolated, but was instead oxidized by peracetic acid to a sulfobutyric acid. The sulfobutyric acid was identified as β-sulfo-γ-hydroxybutyric acid by comparing the properties of its pyridinium salt with those of the authentic material. The reactions involved in these studies are shown in Equation (3). From these data, Yllner postulated that the pure xylo-sulfonic acid had the structure of an α, δ-dihydroxy-γ-sulfovaleric acid.



PRESENTATION OF THE PROBLEM

Because of the difficulties which have been experienced in isolating sulfonated carbohydrates in pure form from spent sulfite pulping liquors of wood, further investigations of the sulfonic acids prepared from simple sugars seemed warranted. Although the relationship of the sulfonic acids from these two different sources is not known, such studies may prove useful as stepping stones to more efficient and effective methods of obtaining and investigating the sulfonated carbohydrates in spent sulfite liquors.

Although the sulfonic acids derived from any of the common wood sugars would have been suitable, those prepared from xylose and arabinose were selected for this study. These two sugars occur commonly in wood, and, being pentoses, the resulting sulfonated products may be less complex in nature than those prepared from hexose sugars. The objectives of this thesis were:

1. To attempt to compare the chemical and physical properties of the products derived from D-xylose with those derived from L-arabinose, and thus to determine the effect of differences in the stereochemistry of the starting sugars on the properties of the resulting sulfonic acids.
2. To attempt to compare the xylo- and arabo-sulfonic acids prepared in this thesis according to a modification of the procedure of Hägglund and co-workers (6) and Adler (7) with the pure xylo-sulfonic acids studied by Yllner (8).

GLOSSARY

1. Crude xylo- and arabo-sulfonic acids: the sulfonic acids prepared form D-xylose and L-arabinose, respectively, by treatment with hot sodium sulfite-bisulfite solutions according to the general procedure of Hägglund and co-workers (6) and Adler (7).

2. Sugar-sulfonic acids: a general term applied to the xylo- and/or arabo-sulfonic acids.

3. Decolorized sugar-sulfonic acids: the xylo- and/or arabo-sulfonic acids after treatment of the crude products with animal charcoal according to the general procedure of Yllner (8). These materials are mixtures of at least three types of compounds.

4. "A" sugar-sulfonic acid: the term applied in this thesis to the first modification of the sugar-sulfonic acids isolated via the brucine salts.

5. "B" sugar-sulfonic acids: the term applied in this thesis to the second modification of the sugar-sulfonic acids isolated via the brucine salts. This material was found to be a mixture.

6. Crude sulfopropionic acid fraction: the materials extracted with 80% ethanol from the residual sodium salts after oxidation of the decolorized sugar-sulfonic acids with chromic acid.

7. EAFW: the chromatographic developer ethyl acetate-acetic acid-formic acid-water (18:3:1:4) (13).

8. BAW: the chromatographic developer n-butanol-acetic acid-water (63:10:27) (13).

9. iBW: the chromatographic developer iso-butyric acid saturated with water (14).

10. PEAm: the chromatographic developer n-propanol-95% ethanol-1.6 N-ammonium carbonate (10:60:30) (15).

11. EAm: the chromatographic developer 95% ethanol-concentrated ammonium hydroxide (100:1) (16).

SUMMARY OF EXPERIMENTAL RESULTS

The sulfonic acids prepared from D-xylose and L-arabinose according to the general procedure of Hägglund and co-workers (6) and Adler (7) were investigated with the following results:

1. The crude xylo- and arabo-sulfonic acids were isolated as barium salts in yields of approximately 47% (as $C_5H_8O_7SBa.H_2O$). The acids were optically inactive as either the barium salts or the free acids. (Found: Crude barium xylo-sulfonate, Ba 37.8, S 9.40; Crude barium arabo-sulfonate, Ba 37.3, S 9.48; Calculated for $C_5H_8O_7SBa.H_2O$, Ba 37.4, S 8.72).

2. The anilides of the crude xylo- and arabo-sulfonic acids separated into four sulfur-bearing spots when chromatographed on Whatman No. 1 paper using EAFW, BAW, and iBW developers.

3. Passing the anilide preparations through a column of Amberlite IR-4B anion-exchange resin[1] in the acetate form resulted in the retention of all sulfur-bearing components by the resin.

4. The crude free xylo- and arabo-sulfonic acids separated into four acidic, sulfur-bearing spots (1, 2, 3, and 4) when chromatographed on Whatman No. 1 paper using developer PEAm. Spots 1 and 2 were shown to contain most of the sulfur-bearing materials. The R_f values of these spots were 0.35, 0.49, 0.58, and 0.64, respectively.

5. Similarly the crude free xylo- and arabo-sulfonic acids separated into four sulfur-bearing spots (1', 2', 3', and 4') when chromatographed on Whatman No. 1 paper using developer EAm. Spots 1' and 2' were shown to contain most of the sulfur-bearing substances.

[1] A product of the Rohm and Haas Company

6. Upon chromatographing individually the crude xylo- and arabo-sulfonic acids on Whatman 3MM paper with EAm developer, spot 3' separated into three acidic spots. Thus, the crude sugar-sulfonic acids separated into a total of six acidic spots in this chromatographic system.

7. Cellulose column chromatography using developer PEAm was not effective in separating the various components in the crude, free xylo- and arabo-sulfonic acids.

8. Decolorizing the crude xylo- and arabo-sulfonic acids with animal charcoal-celite[1] mixtures removed 40 to 50% of the starting material. The resulting barium salts, which were amorphous, were optically inactive.

9. The elemental composition of the decolorized barium xylo- and arabo-sulfonates was $C_5H_8O_7SBa.H_2O$. The infrared spectra of the barium salts were identical.

10. The decolorized xylo- and arabo-sulfonic acids were chromatographically homogeneous using developers PEAm and EAm. This material corresponded to acid spot 2 (or 2') of the crude acids.

11. The decolorized xylo- and arabo-sulfonic acids were separated into two fractions, termed "A" and "B" in this thesis, by fractional crystallization of the brucine salts.

12. The yields of the brucine salt of the "A" fraction of the decolorized xylo- and arabo-sulfonic acids were 20.7 and 16.2%, respectively, on the basis of the decolorized acids. The brucine salt crystals were rod-shaped and analyzed as $C_5H_{10}O_7S.2C_{23}H_{26}O_4N_2$. The specific rotation of the xylo-sulfonic acid salt was -26.4° while that of the arabo-salt was -26.1°.

[1] A product of the Johns Manville Company

The ultraviolet spectra of the two salts were identical as were the infrared spectra.

13. Crystalline barium salts of the "A" fractions were prepared from the brucine salts in yields of about 5% on the basis of the decolorized xylo- and arabo-sulfonic acids. These materials, analyzed as $C_5H_8O_7SBa.H_2O$, were optically inactive, and were not oxidized by periodic acid. The infrared spectra of the two products were identical.

14. The brucine salts of the "B" fraction of the decolorized sugar-sulfonic acids[1] were isolated in a yield of 0.89% on the basis of the decolorized acids. This fraction had needle-shaped crystals and a specific rotation of -36.0°. The brucine salts were converted into amorphous barium salts in a yield of 0.59% on the basis of the decolorized sugar-sulfonic acids. The barium salts, which had a specific rotation of -23.1°, had an infrared spectrum which was similar to that of the decolorized barium sugar-sulfonates. Approximately 0.307 mole of oxidant was consumed per mole[2] of "B" acids during a periodic acid oxidation. Formaldehyde was an oxidation product.

15. Approximately 4 atoms of oxygen were consumed (4.04 atoms with the xylo-acids and 3.96 atoms with the arabo-acids) and an average of about 1.67 moles of carbon dioxide were evolved (1.69 and 1.66 moles, respectively) per mole[2] of decolorized xylo- or arabo-sulfonic acid during the course of a chromic acid oxidation. Sulfosuccinic, and α- and β-sulfopropionic

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1 Combined xylo- and arabo-sulfonic acid brucine salts. The sulfonic acids had been shown to be identical substances.

2 Based on the composition of the acids as $C_5H_{10}O_7S$.

acids were obtained as oxidation products in yields of 0.135, 0.027, and 0.034 mole, respectively, per mole of decolorized sugar-sulfonic acids. 16. Approximately 0.59 mole of oxidant was consumed per mole of decolorized sugar-sulfonic acid[1] during the course of a periodic acid oxidation. Formaldehyde was evolved to the extent of approximately 0.07 mole per mole of decolorized sugar-sulfonic acid. Another oxidation product was shown to be an acidic aldehyde, but it was not identified.

[1] No differentiation was made between the xylo- and arabo-sulfonic acids as they had been shown to be identical substances.

GENERAL METHODS

ANALYTICAL PROCEDURES

SALTS OF METALS

The metal content of salts of acids was determined by the sulfated ash technique (17).

SULFUR

Qualitative tests for sulfur were conducted by the sodium fusion technique (18). Quantitative determinations were made using the Leco procedure (19). In this method, the sample is ignited completely to carbon dioxide, water, and sulfur dioxide in a specially designed, oxygen-fed, Leco furnace. The gases are then conducted to a bubbler tube containing dilute hydrochloric acid, starch, potassium iodide, and sufficient potassium iodate to form a light blue color. As the sulfur dioxide produced in the furnace decolorizes the starch-iodine complex, more iodate is added so that the initial color is present when the combustion reaction has gone to completion. The per cent sulfur of any given weight of sample may be calculated from the amount and normality of potassium iodate consumed. Since this technique was not directly applicable in the case of barium salts due to the formation of barium sulfate, the salts were converted to the free acids by means of Amberlite IR-120 cation-exchange resin[1] in the acid form. Aliquots of the resulting aqueous solution were then tested.

[1] A product of the Rohm and Haas Company

WATER

The amount of water in a compound was determined by oven drying at 150°C. at atmospheric pressure (20).

CARBON AND HYDROGEN

Carbon and hydrogen micro combustion analyses were made by Mr. Robert Krause of the Analytical Laboratory of The Institute of Paper Chemistry (21).

METHOXYL

Methoxyl analyses were made using a modification of Zeisel's method (22).

BRUCINE

Brucine was determined by the general procedure used in the analysis of strychnine sulfate for strychnine (23).

EQUIVALENT WEIGHT

The equivalent weights (or neutral equivalents) of acids were determined by titrating the acid with standard sodium hydroxide to the phenolphthalein end point (18).

DETERMINATION OF SPECTRA

INFRARED

All determinations of infrared spectra were made with a Perkin-Elmer

Model 21 Recording Infrared Spectrophotometer by Mr. Lowell Sell of the Analytical Laboratory of The Institute of Paper Chemistry. The sample was ground with infrared-grade potassium bromide, and the mixture was pressed into a transparent wafer which was placed in the apparatus.

ULTRAVIOLET

The ultraviolet spectra were determined by the Analytical Laboratory of The Institute of Paper Chemistry with a Beckman Model DK-2 Recording Spectrophotometer. The solvent was distilled water.

OPTICAL ACTIVITY

All determinations of optical activity were made on a Zeiss Winkel polarimeter (code word WOLES). Measurements were made at the sodium D-line at ambient temperature using water as the solvent.

CRUDE XYLO- AND ARABO-SULFONIC ACIDS

PREPARATION AND ISOLATION

The crude xylo- and arabo-sulfonic acids were prepared by the general procedure of Hägglund and co-workers (6) and Adler (7). A mixture of 10 grams of D-xylose or L-arabinose, 15 grams of sodium bisulfite, and 20 grams of sodium sulfite were dissolved in sufficient water to make a 200-ml. solution. The initial pH of the liquor ranged from 6.3 to 6.6. The liquor was heated in a stainless steel autoclave to 130°C. in 2-1/2 hours and maintained at that temperature for one additional hour. After cooling, the light yellow colored liquor had a pH of approximately 6.5.

Concentrated sulfuric acid, 12 ml., was added to the liquor slowly and with thorough mixing. The solution was concentrated at 60°C. under reduced pressure until sodium sulfate began to crystallize out. A volume of 25 ml. of 95% ethanol was added, and the resulting mixture was placed in the refrigerator overnight to precipitate more of the sodium sulfate. After removing these salts by filtration, the liquor was diluted to 200 ml. and passed through a column of Amberlite IR-120 in the acid form to remove the remaining sodium ions.

The barium salts were prepared by treating the acid effluent with excess barium carbonate at 80 to 90°C. Upon cooling, the liquor was filtered to remove barium sulfate and excess barium carbonate. The dark red filtrate was concentrated at 60°C. under reduced pressure, and poured into 95% ethanol to precipitate the barium sugar-sulfonates. The tan-colored

salts were removed by filtration, washed with absolute ethanol and ether, and dried at 60°C. under reduced pressure over phosphorus pentoxide. The yields were 46.5 and 47.2% (as $C_5H_8O_7SBa.H_2O$) from xylose and arabinose, respectively. These yields were based on the analysis of a decolorized barium sugar-sulfonate monohydrate (as recorded on page 25).

ANALYSIS

The crude products did not exhibit any optical activity as either the barium salts or the free acids. In contrast to the sulfonic acids derived from glucose (6, 7), the xylo- and arabo-sulfonic acids did not contain a reducing carbonyl group as shown by their inactivity toward phenylhydrazine and Tollens reagent (24). The free acids were oxidized by periodic acid (24). (Found: Crude barium xylo-sulfonate: Ba 37.8, S 9.40; Crude barium arabo-sulfonate: Ba 37.3, S 9.48; Calculated for $C_5H_8O_7SBa.H_2O$: Ba 37.4, S 8.72).

CHROMATOGRAPHIC STUDIES

PAPER CHROMATOGRAPHY

Anilides

The anilides were prepared by heating the crude, free xylo- and arabo-sulfonic acids with aniline on a steam bath until a thick sirup remained. The sirup was dissolved in a small amount of absolute ethanol and spotted on strips of Whatman No. 1 paper. The chromatograms were developed for 44 hours with:

(a) ethyl acetate-acetic acid-formic acid-water (18:3:1:4) (EAFW (13);

(b) n-butanol-acetic acid-water (63:10:27) (BAW) (13); and

(c) isobutyric acid saturated with water (iBW) (14).

After their development, the chromatograms were air dried and sprayed with an acetone solution of Rhodamine B dye. Inspection of the sprayed chromatograms under ultraviolet light showed the anilides as dark areas against a light background (25).

In order to facilitate the identification of sulfur-bearing compounds, a sample of the arabo-sulfonic acids tagged with sulfur 35 (S^{35})[1] was converted into the anilide and chromatographed. The location of sulfur-bearing compounds on the chromatograms was established by noting the radioactive areas with a thin-window G-M counter.

The sulfur-bearing compounds moved very slowly in the above developers and were not cleanly separated. As a very general observation, however, four sulfur-containing spots were discernible.

In an attempt to reduce streaking on the chromatograms, a portion of the anilide preparation was passed through a column of Amberlite IR-4B in the acetate form to remove any unreacted xylo- or arabo-sulfonic acids. Inspection of chromatograms of the effluent showed that all of the sulfur-bearing compounds had been removed.

[1] Sulfur 35 introduced by adding 100 microcuries of radioactive sulfur as sodium bisulfite in 100 microliters of water to a 1/40 scale cook of arabinose. The cook was conducted in a glass tube.

Free Acids

The free acids were prepared by passing an aqueous solution of the crude barium salts through a column of Amberlite IR-120 in the acid form. The resulting acid effluent was concentrated at 60°C. under reduced pressure to a small volume and spotted on strips of Whatman No. 1 paper. The chromatograms were developed with:

(a) n-propanol-95% ethanol-1.6N-ammonium carbonate (10:60:30) (PEAm) (15) and

(b) 95% ethanol-concentrated ammonium hydroxide (100:1) (EAm) (16).

Development required 24 hours using PEAm and 72 hours using EAm. After their development, the sheets were air dried. The chromatograms were sprayed with 4% formaldehyde and dried at 105°C. to convert the ammonium salts to urotropine and the free acids (26) which were then detected as red spots against a yellow-orange background by spraying with a 0.1% aqueous solution of methyl orange.

Using either developer PEAm or EAm, four acid spots were discernible. Using PEAm, these spots had R_f values of 0.35, 0.49, 0.58 and 0.64 and were termed acids 1, 2, 3 and 4, respectively. R_f values could not be assigned using developer EAm since the solvent ran off the bottom of the sheets; but the spots were termed acids 1', 2', 3', and 4' in the order of increasing distance from the origin.

In the preliminary phases of this work, arabo-sulfonic acids tagged with S^{35} were chromatographed to facilitate the identification of sulfur-

containing materials. Acids 1 and 1' were very rich in sulfur; acids 2 and 2' also contained significant quantities; but acids 3 and 4, and 3' and 4' contained much less than the others.

CELLULOSE COLUMN CHROMATOGRAPHY

Attempts were made to separate the components in the crude xylo- and arabo-sulfonic acids on chromatographic columns (2 by 20 in.) packed with Whatman cellulose powder. Some columns were packed dry, and others were wet packed using a slurry of cellulose powder in acetone. In the latter case, the acetone was then progressively replaced by acetone-ethanol, ethanol, and PEAm mixtures. The free acids, one gram, were applied to the top of the column in concentrated aqueous or alcoholic solutions and washed into the cellulose with small portions of the PEAm developer. Solvent PEAm was used to separate the components. The effluent from the column was divided into fractions which were, in turn, monitored by paper chromatography using the techniques described previously. (See Page 21. The separation of the various components in the crude xylo- and arabo-sulfonic acids were found to be relatively poor so that this technique was abandoned.

HEAVY PAPER CHROMATOGRAPHY

The behavior of the crude sugar-sulfonic acids on Whatman 3MM paper using developer EAm was studied next. Upon developing for 72 hours, the spot previously termed 3' separated into three spots. Thus, in this chromatographic system, the crude acids separated into a total of six

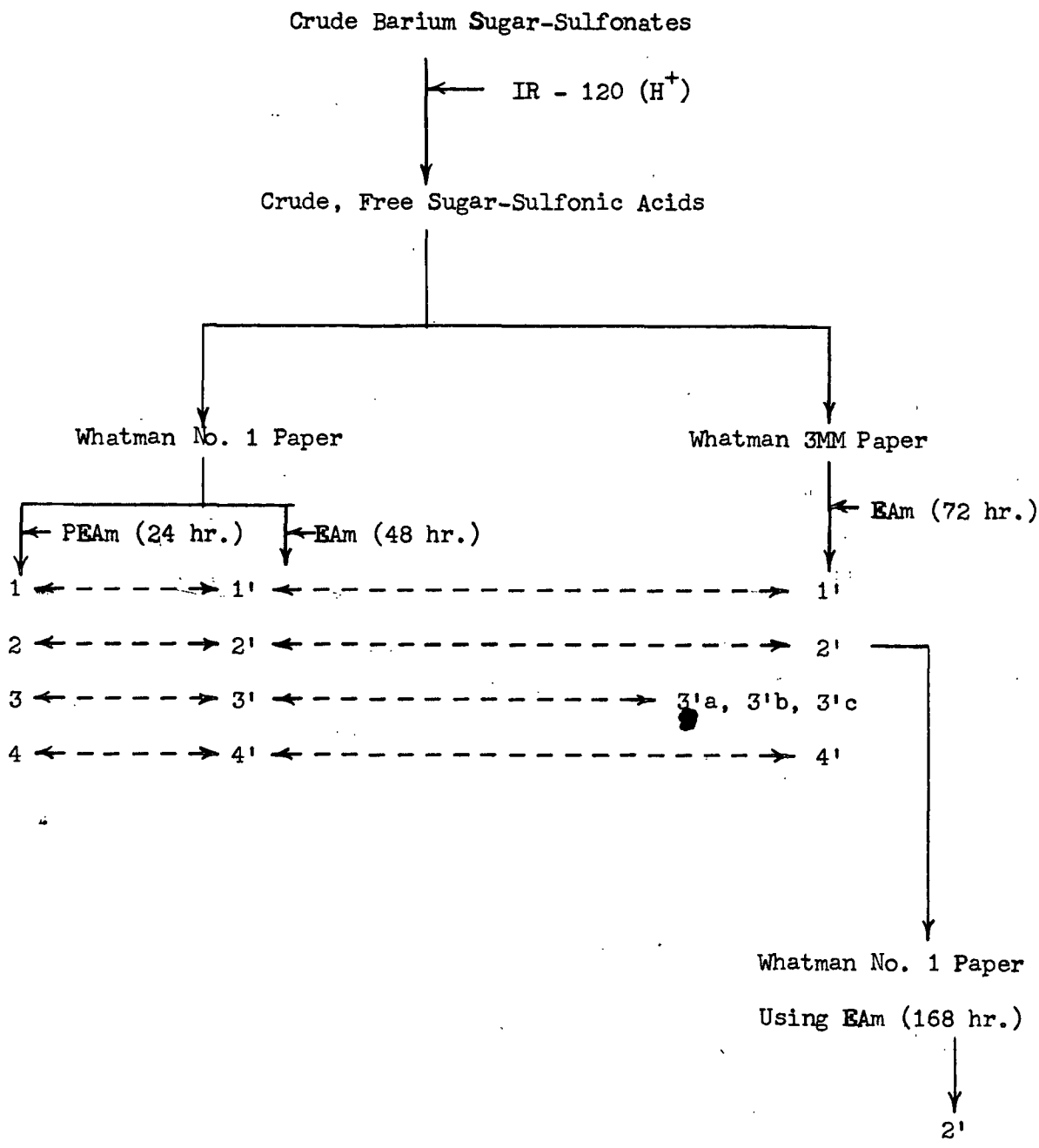


Figure 1

Chromatographic Studies of the Crude, Free Sugar-Sulfonic Acids

acidic spots, termed 1', 2', 3'a, 3'b, 3'c, and 4'. The relationship between the acid spots as found in the various chromatographic systems is shown diagrammatically in Figure 1.

Although no determinations of the actual quantities of the various components in the crude sugar-sulfonic acids were made, the following estimates were made on the basis of the chromatographic studies: components 4' and 3'c were present in only very small amounts; components 3'b and 3'a were present in about equal quantities but still constituted only a small portion of the total acids; components 2' and 1', which contained most of the sulfur-bearing materials, were present in about equal quantities and constituted the major portion of the crude xylo- and arabo-sulfonic acids.

Since spot 2' was one of the more important sulfur-bearing spots, it was eluted from several chromatograms and rechromatographed, but this material remained chromatographically homogeneous even upon being developed for 168 hours.

DECOLORIZED XYLO- AND ARABO-SULFONIC ACIDS

PREPARATION

The decolorized barium salts were prepared from the crude products by treatment with animal charcoal-celite (1:1) in yields of from 50 to 60% according to the method of Yllner (8). The resulting products were white to buff colored in the solid state and formed yellow aqueous solutions.

ANALYSIS

The decolorized salts could not be crystallized and were optically inactive. The infrared spectra of the decolorized barium xylo- and arabo-sulfonates were identical and are shown in Figure 2. The elemental compositions are given in Table I.

TABLE I

DECOLORIZED BARIUM XYLO- AND ARABO-SULFONATES

Component	Calculated for $C_5H_8O_7SBa.H_2O$, %	Found	
		Xylo-Acid Salt, %	Arabo-Acid Salt, %
Ba	37.4	37.5	37.6
S	8.73	9.08	8.85
C	16.4	16.1	16.3
H	2.18	2.46	2.48
H_2O	4.90	4.97	4.90

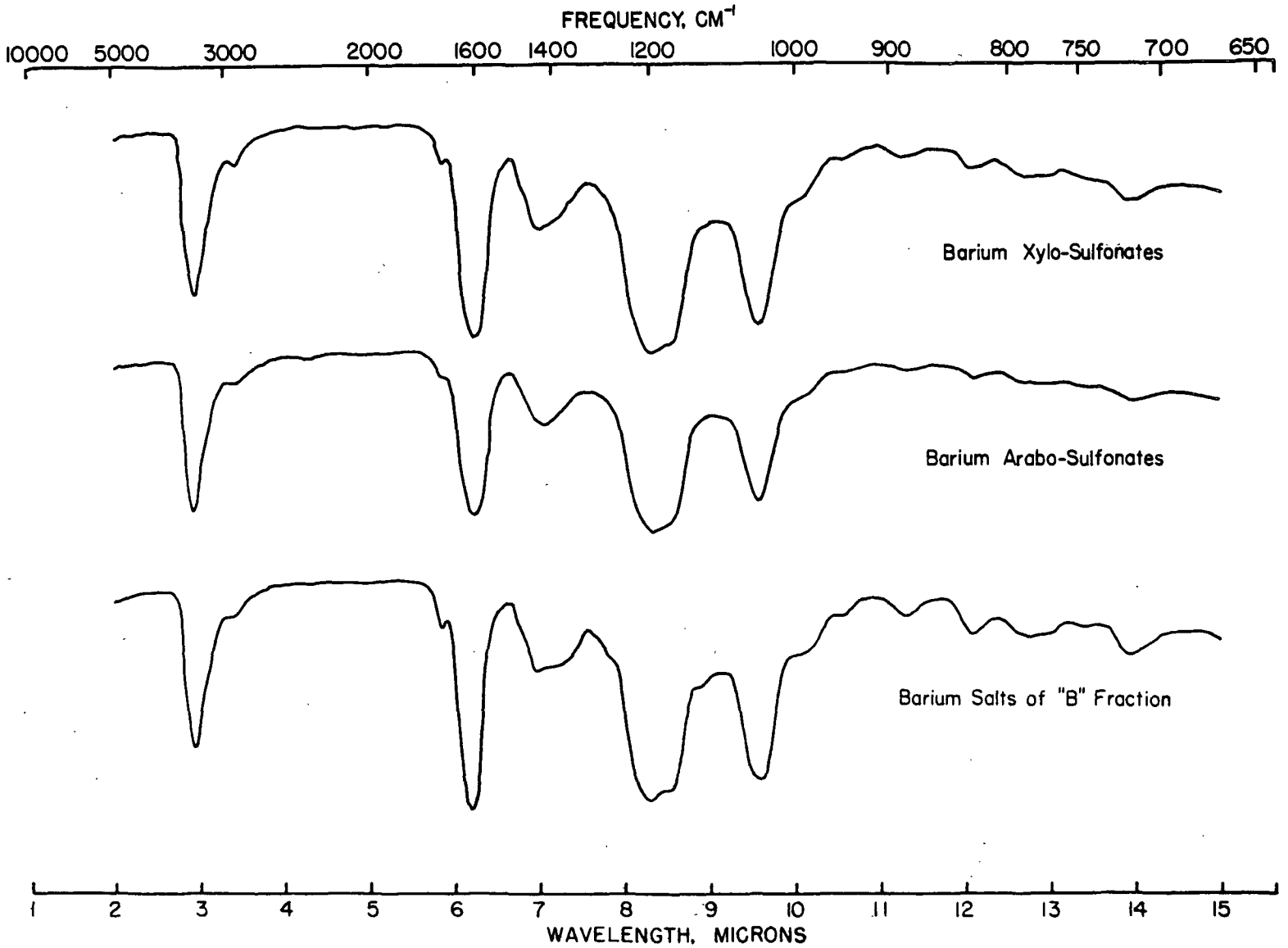


Figure 2. Infrared Spectra of the Decolorized Barium Sugar-Sulfonates

CHROMATOGRAPHIC STUDIES

The free decolorized xylo- and arabo-sulfonic acids were chromatographed on Whatman No. 1 paper using developers PEAm and EAm. The acid spots were detected by the formaldehyde-methyl orange spray technique (See Pages 21 and 22). Only one acid spot, corresponding to acid 2 (or 2') of the crude xylo- or arabo-sulfonic acids, was found, thus indicating that the material was chromatographically homogeneous.

FRACTIONATION

Two fractions of the decolorized xylo- and arabo-sulfonic acids were isolated via the brucine salts by a modification of Yllner's technique (8). The brucine salts were prepared by treating the free sugar-sulfonic acids with an excess of brucine and heating at 70°C. for 1/2 hour. After cooling and extracting the excess brucine with chloroform, the aqueous solutions of the brucine salts were concentrated at 60°C. under reduced pressure to sirups. Acetone was added to the hot, concentrated aqueous solutions to incipient opalescence. Upon cooling, the brucine salts crystallized.

The first crystalline products were recrystallized twice from water-acetone mixtures. The resulting substances, which were white in color and rod-shaped, were called the "A" fractions by the present author. The yields were 20.7 and 16.2% on the basis of the decolorized xylo- and arabo-sulfonic acids, respectively. The optical activities were $[\alpha]_D^{24.8} = -26.4° \pm 0.2°$ (c 4.92, 2 dm. tube, water) and $[\alpha]_D^{25.0} = -26.1° \pm 0.2°$ (c 4.97, 2

dm. tube, water), respectively. The ultraviolet spectra of the two salts were identical and had transmittance maxima at 236 and 285 mμ and minima at 263 and 300 mμ. In addition, the infrared spectra were identical as shown in Figure 3.

Since the above data, together with the information which was obtained on the decolorized products, indicated that the xylo- and arabosulfonic acids were identical substances, the mother liquors from the "A" fraction brucine salts were combined and subjected to further fractional crystallization. A second modification of the brucine salts, termed the "B" fraction in this thesis, was obtained in a yield of 0.89% on the basis of the initial decolorized sugar-sulfonic acids. The "B" fraction brucine salts were needle-shaped and had a specific rotation of $[\alpha]_D^{24.6} = -36.0° \pm 0.8°$ (c 3.04, 2 dm. tube, water).

The "A" and "B" brucine salts were converted into the barium salts as follows: An aqueous solution of the brucine salt was treated with ammonium hydroxide until the solution was strongly alkaline to alkacid test paper.[1] After extracting the liberated brucine with chloroform, the aqueous solution of ammonium salts was passed through a column of Amberlite IR-120 in the acid form. The barium salt was formed by treating the acid effluent with barium carbonate or hydroxide. The salt solution was then concentrated to a small volume at 60°C. under reduced pressure. The barium salts of the "A" fractions crystallized from the concentrated aqueous solutions upon the addition of 95% ethanol. These materials were

[1] A product of Fisher Scientific Company.

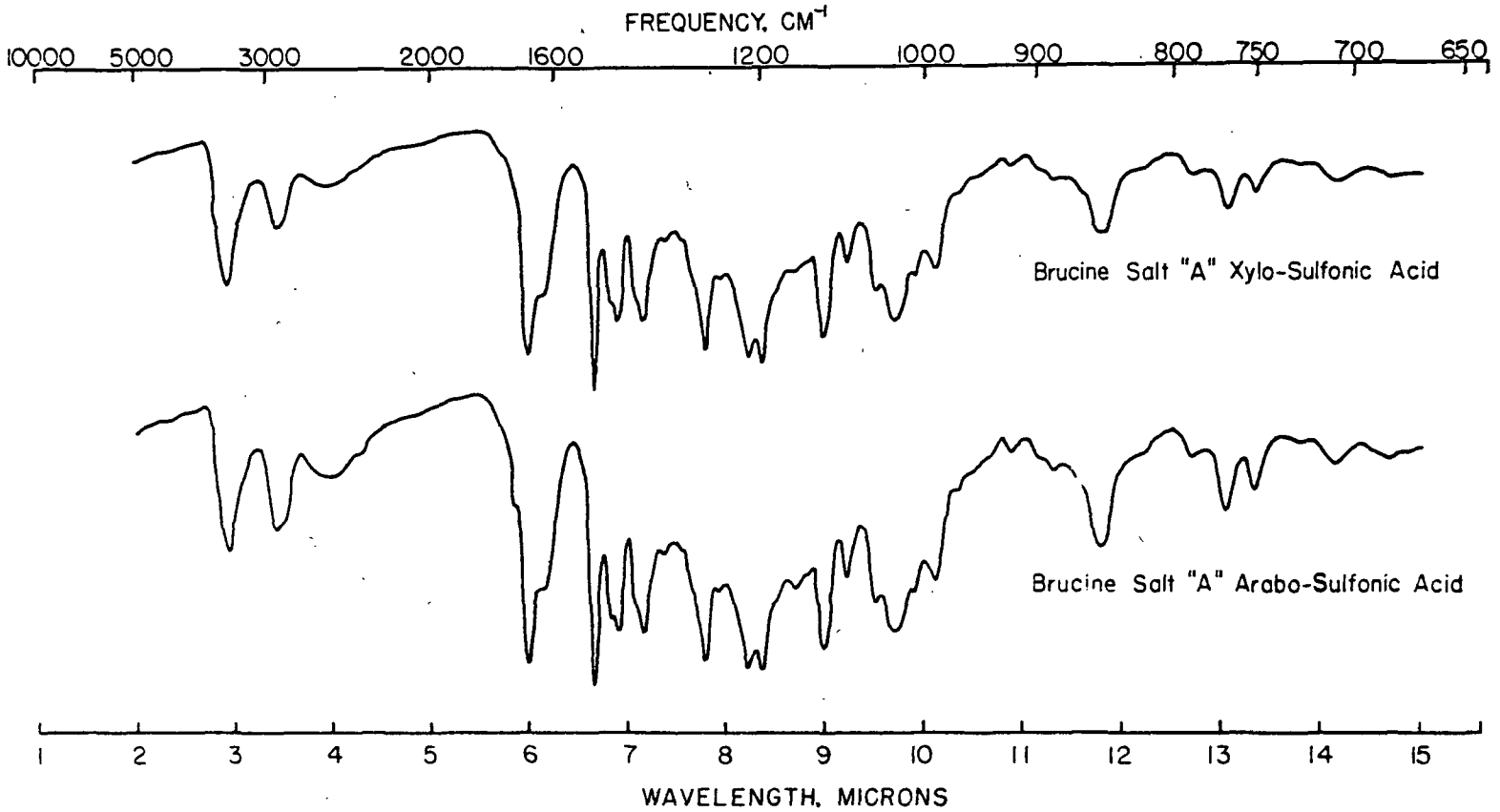


Figure 3. Infrared Spectra of the Brucine Salts of the "A" Sugar Sulfonic Acids.

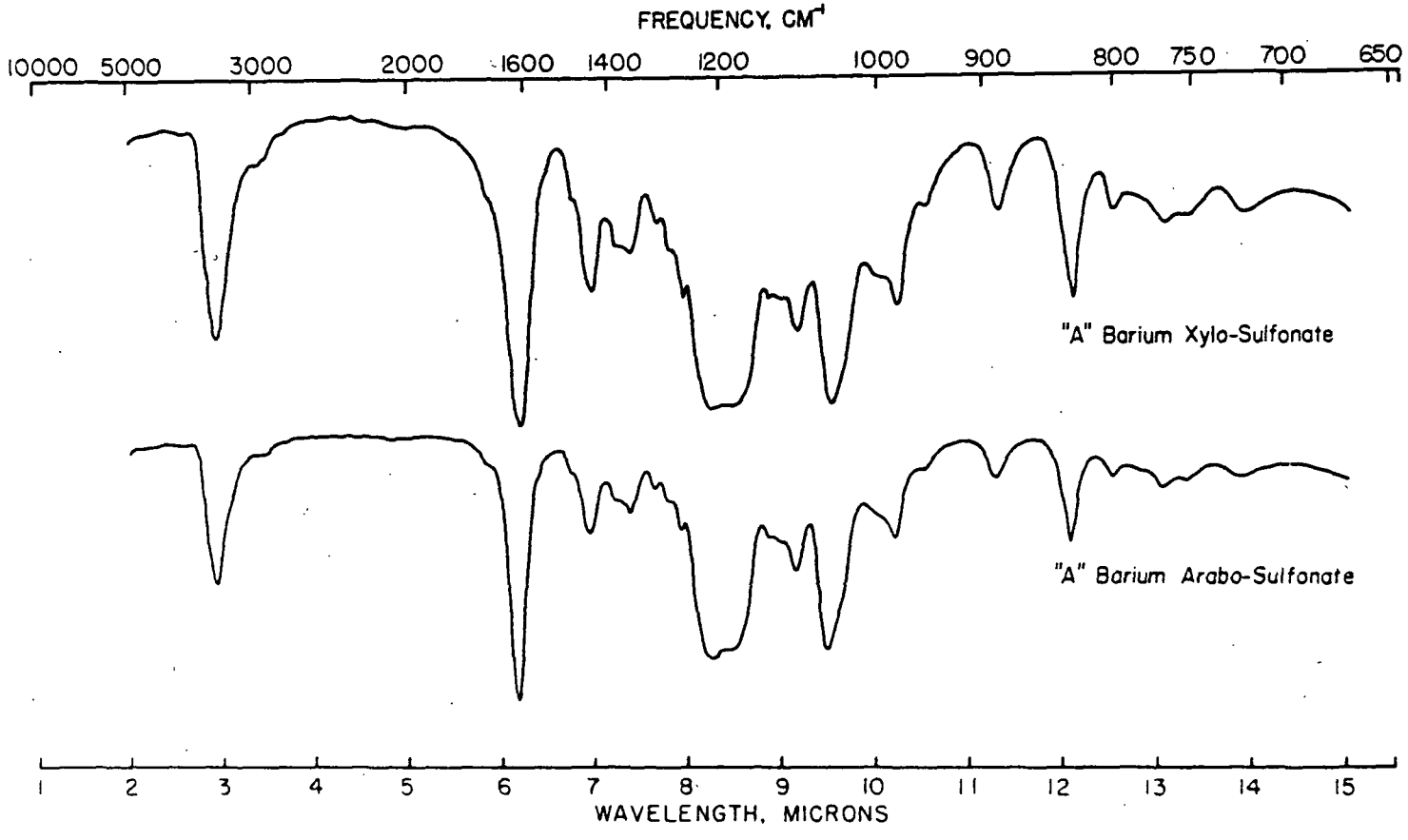


Figure 4. Infrared Spectra of the "A" Barium Sugar-Sulfonates.

recrystallized once from water-ethanol. The yields were 24.2 and 31.1% based on the brucine salts or 5.04 and 5.03% based on the initial decolorized xylo- and arabo-sulfonic acids, respectively. The barium salt of the "B" fraction could not be crystallized and was, therefore, isolated by precipitation in ethanol in a yield of 65.5% based on the brucine salt or 0.59% based on the initial decolorized sugar-sulfonic acids.

The "A" acids were optically inactive as either the barium salts or the free acids. When the free acids were chromatographed on Whatman No. 1 paper using developers PEAm or EAm, only one acidic spot, corresponding to spot 2 or 2', respectively, of the crude acid mixtures, was found. The infrared spectra of the "A" barium xylo- and arabo- sulfonates were identical as shown in Figure 4. The free acids were stable toward periodate.

The optical activity of the barium salt of the "B" fraction was $[\alpha]_D^{24.0} = -23.1° \pm 0.6°$ (c 1.74, 2 dm. tube, water), and its infrared spectrum was similar to that of the decolorized barium sugar-sulfonates as shown in Figure 2. The free acid was slowly oxidized by periodate indicating the presence of a 1,2-glycol group (0.307 mole periodate consumed per mole of "B" acid in 72 hours). The consumption of oxidant was followed iodometrically. A qualitative test with chromotropic acid indicated that formaldehyde was one of the oxidation products (27).

The chemical analyses of the "A" fractions are given in Table II. The "B" fraction, however, was not analyzed.

TABLE II

ANALYSES OF THE "A" FRACTION OF THE XYLO- AND ARABO-SULFONIC ACIDS

	Component	Calculated	Found	
		Value, %	Xylo-Acid Salt, %	Arabo-Acid Salt, %
Brucine salt	S	3.20	3.08	3.06
$C_5H_{10}O_7S.2C_{23}H_{26}O_4N_2$	OCH_3	12.4	12.2	12.0
	Brucine	79.0	76.5	75.8
Barium salt	Ba	37.4	37.8	37.6
$C_5H_8O_7SBa.H_2O$	S	8.72	8.35	8.46

CHROMIC ACID OXIDATIONS

INTRODUCTION

The data which were collected on the "A" and "B" fractions of the decolorized xylo- and arabo-sulfonic acids established the fact that the decolorized acids were a mixture of several types of compounds. The elemental analyses indicated, however, that both the decolorized and "A" acids had the composition $C_5H_{10}O_7S$. Although the methods of preparation and isolation of the sugar-sulfonic acids studied in this thesis were significantly different from those employed by Yllner (8), the data indicated but did not prove that the "A" acids and the pure xylo-sulfonic acid studied by Yllner were probably the same substance, and, therefore, possibly had the structure of an α, δ-dihydroxy-γ-sulfovaleric acid.

Based upon this assumption together with Yllner's assumption that chromic acid oxidation of α, δ-dihydroxy-γ-sulfovaleric acid produced only sulfosuccinic acid, it seemed possible to learn about the nature of the mixture of components in the decolorized sugar-sulfonic acids by studying the chromic acid oxidation products. Furthermore, if sulfosuccinic acid were not a product of the oxidation, one or both of the above assumptions would have been proven false.

PRELIMINARY INVESTIGATIONS

In the initial work, the decolorized barium xylo- and arabo-sulfonates were oxidized with chromic acid using essentially the same reaction conditions employed by Yllner (8) as shown in Table III. The consumption of oxidant was found to be approximately 4 atoms of oxygen per $C_5H_{10}O_7S$ unit. This value was in accord with Yllner's hypothetical reaction of α, δ-dihydroxy-γ-sulfovaleric acid to yield sulfosuccinic acid (rate of oxidation curves are given in Figure 5). However, approximately 1.5 moles of carbon dioxide were evolved per $C_5H_{10}O_7S$ unit instead of the theoretical one mole per $C_5H_{10}O_7S$ unit. In addition, little or no sulfosuccinic acid was isolated from the oxidation liquor.

The consumption of oxidant was determined iodometrically using the general procedure of Yllner (8), and the method used to measure the quantity of carbon dioxide evolved is discussed in Appendix I.

To determine whether sulfosuccinic acid was stable toward the oxidizing medium, the acid was synthesized according to the procedure of

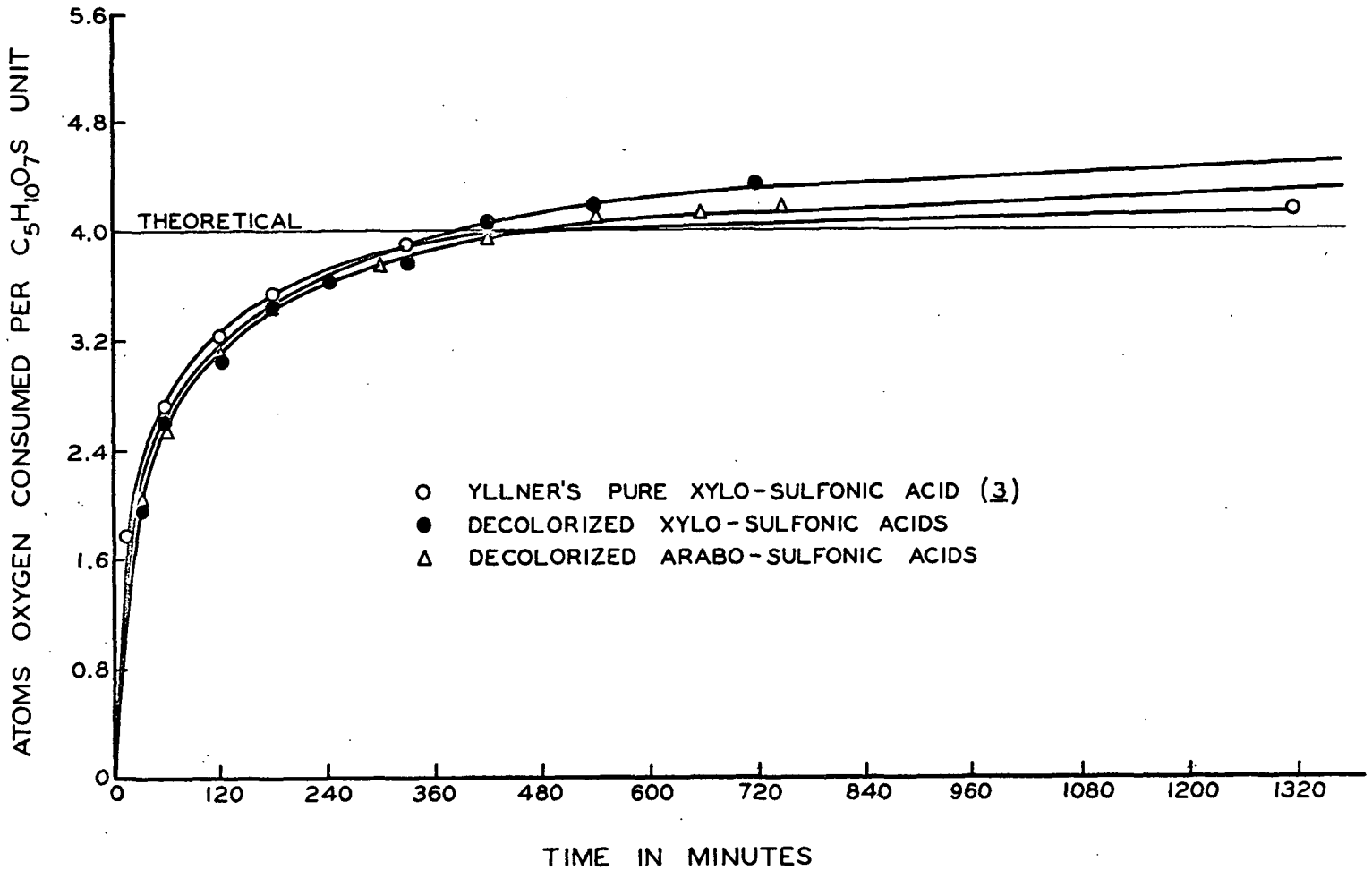


Figure 5. Chromic Acid Oxidation of the Sugar-Sulfonic Acids

TABLE III

REACTION CONDITIONS OF THE CHROMIC ACID OXIDATIONS OF THE DECOLORIZED SUGAR-SULFONIC ACIDS

	Original Conditions (23)		Modified Conditions	
Component	Weight, g.	Molar Ratio	Weight, g.	Molar Ratio
Sugar-Sulfonic Acid	0.0975	1	0.585	1
$K_2Cr_2O_7$	0.50	11.2 (as [O])[a]	3.0	11.2 (as [O])[a]
H_2SO_4	4.41	100	5.35	20

Volume of solution made up to 50 ml. with water

[a] Calculated from the following equations:

$$K_2Cr_2O_7 \rightleftharpoons K_2O.2CrO_3$$
$$2CrO_3 \rightarrow Cr_2O_3 + 3[O]$$

Backer and van der Zanden (28) (See Appendix II), and subjected to chromic acid oxidation under the conditions which would exist if all the decolorized sugar-sulfonic acids were converted into this product. The sulfosuccinic acid was found to be stable with regard to both further oxidation (less than 0.1 atom oxygen consumed per mole of sulfosuccinic acid) and decarboxylation (0.03 mole carbon dioxide evolved per mole sulfosuccinic acid).

The efficiency of the process which had been used in attempting to isolate sulfosuccinic acid from the oxidation liquors of the decolorized sugar-sulfonic acids was found to be very low. Facsimiles of the oxidation liquors were prepared containing known amounts of authentic sulfosuccinic acid, and the recovery was noted. It was found that the major loss of

sulfosuccinic acid occurred during the removal of the large quantities of sulfate ion by precipitation as barium sulfate.[1]

On the basis of this information, the composition of the oxidation liquor was modified (from Yllner's conditions) to reduce the amount of sulfuric acid required in the reaction. The altered liquor composition is shown in Table III. The reactions were run at 100°C. and required approximately 24 hours to go to completion.

Under these modified conditions, the consumption of oxidant remained near the value of 4 atoms of oxygen (4.04 and 3.96 atoms for the xylo- and arabo-acids, respectively) but 1.67 moles of carbon dioxide (1.69 and 1.66 moles, respectively) were evolved per $C_5H_{10}O_7S$ unit. Authentic sulfosuccinic acid was found to be stable under these modified conditions of the oxidation.

OXIDATION PRODUCTS

By studying facsimiles of the oxidation liquors containing known amounts of authentic sulfosuccinic acid, a process was developed whereby a recovery of 50% could be consistently effected. When this process was applied to the oxidation liquors of the decolorized xylo- and arabo-sulfonic acids, the yields of sulfosuccinic acid were 12.3 and 14.7%, respectively, based on the total conversion of the sugar-sulfonic acids (as $C_5H_{10}O_7S$) to sulfosuccinic acid. This recovery procedure is discussed in detail in Appendix III. The acid was identified by comparison of the

[1] Attempts to separate sulfosuccinic and sulfuric acids on anion-exchange resin columns were unsuccessful.

infrared spectrum of its barium salt to that of the authentic material and by its elemental analysis.

In addition to sulfosuccinic acid, α- and β-sulfopropionic acids were also shown to be oxidation products of the decolorized xylo- and arabo-sulfonic acids. The isolation of these acids is given in Appendix IV. The yields of α-sulfopropionic acid were 2.9 and 2.6% based on the total conversion of the decolorized xylo- and arabo-sulfonic acids, respectively, to this material. Similarly, the yields of β-sulfopropionic acid were 3.1 and 3.7%, respectively. The sulfopropionic acids were identified by comparing the infrared spectra of their barium salts with those of the authentic sulfopropionates. Authentic barium α-sulfopropionate was synthesized by the method of Backer and Dubsky (29) (see Appendix V), while the β isomer was prepared according to the procedure of Kharasch, Chao, and Brown (30) (see Appendix VI). The S-benzyl-iso-thiourea salt of the β-isomer isolated from the oxidation liquors was prepared (31) and found to melt at 151 to 152°C.[1] Its melting point was not depressed when mixed with the salt of the authentic acid.

PERIODIC ACID OXIDATION

An amount of 1.2 gram (1/3 mole fraction) of the decolorized xylo- or arabo-sulfonic acids was slowly oxidized by 2.16 grams (2/3 mole fraction) of periodic acid in 100 ml. of solution at room temperature and in the absence of light. The consumption of oxidant, which was followed

[1] Uncorrected.

iodometrically, was found to be 0.59 mole per $C_5H_{10}O_7S$ unit. An attempt was made to follow the evolution of aldehydes by alkaline hypoiodite oxidation (32). However, when the decolorized sugar-sulfonic acids alone were subjected to alkaline hypoiodite oxidation, it was found that extensive oxidation occurred (1.49 moles hypoiodite consumed per mole of sugar-sulfonic acid). The nature of this oxidation is difficult to envision since the sugar-sulfonic acids did not contain any aldehyde groups. Hence, it was apparent that this reaction was unsuitable for following the evolution of aldehydes during the periodic acid oxidations.

The periodic acid oxidation was repeated, and in addition to following the consumption of oxidant, the evolution of formaldehyde was determined colorimetrically with chromotropic acid (33) and gravimetrically as formaldehyde dimethone (34). The amount of formaldehyde evolved was found by the colorimetric method to be approximately 0.15 mole per mole of periodic acid consumed and 0.12 mole per mole by the gravimetric procedure. In the case of the latter procedure, the crystalline product melted at 187 to 189°C.,[1] and its melting point was not depressed when mixed with an authentic sample of formaldehyde dimethone.

Another oxidation product was isolated as follows: An aliquot of the reaction liquor was neutralized with barium hydroxide to precipitate the insoluble barium periodate and iodate salts. After filtering, the liquor was poured into 95% ethanol to precipitate the water-soluble barium salts, and, thereby, separate them from the formaldehyde. The solids were removed by filtration, washed with absolute ethanol and

[1] Uncorrected.

ether, and dried at 60°C. under reduced pressure over phosphorus pentoxide. An aqueous solution of the barium salts formed a precipitate with 2,4-dinitrophenylhydrazine and reacted with Tollens reagent (24) indicating the presence of an aldehyde group.

DISCUSSION

INTRODUCTION

Yllner's work on the sulfonic acids derived from xylose (8) has been discussed briefly in the Historical Review section of this report. The differences between the methods used in this thesis and in Yllner's studies for preparing and isolating the crude sugar-sulfonic acids may be summarized as follows:

1. Liquor Composition (Applied chemical expressed as molar ratio) -- Yllner: xylose 1, sodium pyrosulfite 2.34 (expressed as bisulfite, $S_2O_5^{=} + H_2O \rightarrow 2HSO_3^-$), sodium sulfite 2.34; Thesis: xylose or arabinose 1, sodium bisulfite 2.88, sodium sulfite 3.57. Both preparations were conducted in aqueous solutions at pH 6.3-6.6.
2. Reaction Vessel -- Yllner: glass; Thesis: stainless steel.
3. Heating Schedule -- Yllner: one hour at 135°C.; Thesis: 2-1/2 hours from room temperature to 130°C., one additional hour at 130°C.
4. Isolation of Crude Barium Sugar-Sulfonates -- Yllner, crude salts divided into four fractions by ethanol precipitation; Thesis, no attempt to fractionate the crude salts.

Because of these differences, it was not possible to predict that the sugar-sulfonic acids prepared during the thesis work were identical with those of Yllner. However, many similarities between the products have been found as will be shown in the following discussion.

CRUDE XYLO- AND ARABO-SULFONIC ACIDS

The crude barium xylo- and arabo-sulfonates[1] contained barium and sulfur in ratios of 0.94 and 0.92, respectively. This ratio would be one for a pure sulfo-carboxylic acid. Calculated as $C_5H_8O_7SBa.H_2O$ (based on the analysis of the decolorized barium sugar-sulfonates), the yields were 46.5 and 47.2%, respectively. These yields were in general agreement with Yllner's yield of approximately 50%. The fate of the other one-half of the starting sugar was not investigated. The crude acids contained some material with a 1,2-glycol grouping as shown by their reactivity toward periodate. In agreement with Yllner's investigations of his xylo-sulfonic acids, the crude sugar-sulfonic acids were optically inactive as either the free acids or the barium salts. The particular significance of this fact is discussed in the Formation of the Sugar-Sulfonic Acids division of the Discussion section of this report.

The crude xylo- and arabo-sulfonic acids exhibited identical chromatographic properties in the various systems employed in this thesis.

In the initial phases of the chromatographic studies, the crude acids were converted into the anilide derivative and studied according to a paper chromatographic procedure suggested by Green (25). Inspection of the resulting chromatograms indicated that at least four sulfur-bearing materials were present in both sulfonic acid preparations; however, the separation of these components was poor because of extensive streaking.

[1] Unless otherwise stated, the sugar-sulfonic acids referred to are those studied in the thesis investigations.

In an attempt to reduce the streaking, the anilide preparations were passed through a column of Amberlite IR-4B in the acetate form to remove any unreacted sugar-sulfonic acids which may have been present. Chromatograms of the resulting effluent showed that all sulfur-bearing materials had been retained by the resin.

The chromatographic behavior of the free xylo- and arabo-sulfonic acids on Whatman No. 1 paper was studied next. The sulfonic acids produced identical chromatograms, and at least four acidic, sulfur-bearing spots were found on the sheets. These spots were termed 1, 2, 3, and 4 using developer PEAm and 1', 2', 3', and 4' using developer EAm. Most of the sulfur-bearing materials were found in spots 1 and 2 or 1' and 2'. In the case of the xylo-sulfonic acid studied by Yllner, the crude barium salts of all four fractions were converted into the ammonium salts and chromatographed using ethanol-water (4:1) as the developer. Fractions 1, 2, and 3 gave similar results with one main spot and several minor spots. Fraction 4, however, separated into a total of five almost equally strong spots.

In an attempt to isolate larger quantities of the various materials in the crude acids, the acids were chromatographed on columns of cellulose powder using developer PEAm. The effluent was monitored by paper chromatography using both PEAm and EAm developers. Because of the relatively poor separation of the components in the crude acid mixtures, this technique was abandoned.

The chromatographic behavior of the crude xylo- and arabo-sulfonic

acids on Whatman 3MM paper using developer EAm was studied next. Using this chromatographic system, the spot previously termed 3' (on Whatman No. 1 paper using EAm developer) separated into three spots. The separation of this spot was noticeable in this system probably because of the greater sample size which can be applied to Whatman 3MM paper than can be used on Whatman No. 1 paper. Spot 2', one of the important sulfur-bearing components, was found to be chromatographically homogeneous using developers PEAm and EAm, however. Thus, by this technique, a total of at least six different substances had been found in the crude xylo- and arabo-sulfonic acids.

DECOLORIZED XYLO- AND ARABO-SULFONIC ACIDS

PREPARATION AND ANALYSIS

The decolorized xylo- and arabo-sulfonic acids were prepared from the crude products by treatment with animal charcoal-celite mixtures according to the general procedure of Yllner (8). Approximately one-half of the materials in the crude acids was removed by this treatment.

Paper chromatograms of the decolorized xylo- and arabo-sulfonic acids were identical. Only one acid spot, corresponding to spot 2 or 2' of the crude acids, was found using developer PEAm or EAm, respectively. Yllner's charcoal-purified xylo-sulfonic acids were chromatographically homogeneous also.

The decolorized xylo- and arabo-sulfonic acids were optically inactive and analyzed as $C_5H_{10}O_7S$ in agreement with Yllner's findings on his

decolorized xylo-sulfonic acids. The infrared spectra of the decolorized barium xylo- and arabo-sulfonates were identical, indicating that the acids were identical substances.

FRACTIONATION VIA THE BRUCINE SALTS

Based on the analytical data and spectra which were obtained on the crystalline brucine and barium salts, the "A" fractions of the xylo- and arabo-sulfonic acids were shown to be identical substances. The "A" acids analyzed as $C_5H_{10}O_7S$, were optically inactive, and were stable toward periodate. The corresponding fraction of the xylo-sulfonic acids investigated by Yllner exhibited the same properties. In addition, the crystalline structures of the corresponding brucine and barium salts were identical. These data indicate but do not prove that the "A" xylo- and arabo-sulfonic acids prepared in the thesis investigations were identical with the pure xylo-sulfonic acid studied by Yllner. Although the methods of preparing and isolating the crude sugar-sulfonic acids differed to some degree, it appeared possible that the resulting products prepared in this thesis and by Yllner were identical substances. Yllner showed that the pure xylo-sulfonic acid he isolated via its brucine salt probably had the structure of an α, δ-dihydroxy-γ-sulfovaleric acid.

The "B" fraction of the sugar-sulfonic acids was isolated via its brucine salt from the mother liquors of the brucine salt of the "A" fraction. This material, upon conversion to an amorphous barium salt, was levo-rotating in aqueous solution, and its infrared spectrum was similar to that of the initial decolorized barium sugar-sulfonates. This material

was a mixture of substances as shown by the fact that it was oxidized by periodate in less than a stoichiometric amount; that is, 0.307 mole periodate consumed per mole (based on $C_5H_{10}O_7S$ composition) of "B" sugar-sulfonic acid. A qualitative test of the periodate oxidation liquor indicated that formaldehyde was one of the oxidation products so that some component in the "B" acids contained a terminal 1,2-glycol.

The fact that the "B" acids consumed only approximately one-half as much periodate as did the decolorized sugar-sulfonic acids indicated that the "B" acids were enriched with the periodate-stable "A" acid. However, if this were true, it would seem that the infrared spectrum of the "B" acids would be more nearly identical with that of the "A" acids rather than that of the decolorized sugar-sulfonates as was actually found. Assuming that the periodate data were correct, a possible explanation of this conundrum may be that the ability of infrared spectral analysis to detect changes in the composition of mixtures of substances is at least partially masked in the case of noncrystalline materials. Referring to the spectra of the decolorized (see Figure 2, page 26) and "A" (see Figure 4, page 30) barium sugar-sulfonates, the spectra were similar but not identical. In general, the absorption bands of the spectra of the amorphous decolorized salts were wider, and, therefore, less distinctive than were those of the crystalline "A" salts. Investigations have been conducted on other compounds to show the differences in the spectra of crystalline and noncrystalline forms of the same compound, and similar effects have been noted; that is, the spectrum of a substance in noncrystalline form is much less distinctive than the spectrum of the same material in

crystalline form (35). Thus, while there were differences in the composition as well as crystallinity of the three fractions of the sugar-sulfonic acids subjected to infrared spectral analysis, this phenomenon may at least partially account for the similarity of the spectra of the decolorized and "B" barium sugar-sulfonates.

Yllner also isolated a second modification of the brucine salts of his xylo-sulfonic acid with the same crystalline form as the "B" acid brucine salts. The yield of this material as reported by Yllner was much greater than in the thesis investigations. Although Yllner did not investigate the nature of this second modification of his xylo-sulfonic acids, he suggested that it might consist of a second pair of d,l isomers of α, δ-dihydroxy-γ-sulfovaleric acid.

CHROMIC ACID AND PERIODIC ACID OXIDATIONS

Chromic acid oxidation was used by Yllner as a part of the investigations to determine the structure of the pure xylo-sulfonic acid which was isolated via its brucine salt. The postulated reaction is shown in Equation (2). The consumption of oxidant was found to be nearly equal to that theoretically required to produce sulfosuccinic acid, but no attempt to isolate the product was reported (8).

In this thesis, the decolorized xylo- and arabo-sulfonic acids were oxidized with chromic acid in an attempt to determine the nature of the mixture of the sugar-sulfonic acids by studying the reaction and reaction products. The consumption of oxidant was found to be nearly equal to the

value reported by Yllner, but 1.67 moles of carbon dioxide per $C_5H_{10}O_7S$ unit were evolved under the modified conditions of reaction as shown in Table III. Authentic sulfosuccinic acid, prepared according to the method of Backer and van der Zanden (28), was stable toward the modified oxidizing medium.

Investigations of the oxidation liquors showed that sulfosuccinic, and α- and β-sulfopropionic acids were oxidation products. The yields of these materials were approximately 0.135, 0.027, and 0.034 mole, respectively, per mole of decolorized sugar-sulfonic acid (on $C_5H_{10}O_7S$ basis).

These results were not of significant value in determining the nature of the mixture of acids in the decolorized sugar-sulfonic acid since analysis of potential reaction routes showed that all the data would be satisfied if but one substance, α, δ-dihydroxy-γ-sulfovaleric acid, were considered as shown in Figure 6. However, other hypothetical isomers of $C_5H_{10}O_7S$ composition can also satisfy these same data so that it is impossible to make positive statements regarding the composition of the decolorized sugar-sulfonic acids on the basis of this data alone. However, since the reaction and reaction products were essentially the same for both the decolorized xylo- and arabo-sulfonic acids, the fact that the acids derived from the two different sugars were identical substances was demonstrated still further.

Although the chromic acid oxidations were not useful in diagnosing the nature of the decolorized sugar-sulfonic acids, the periodate oxidations

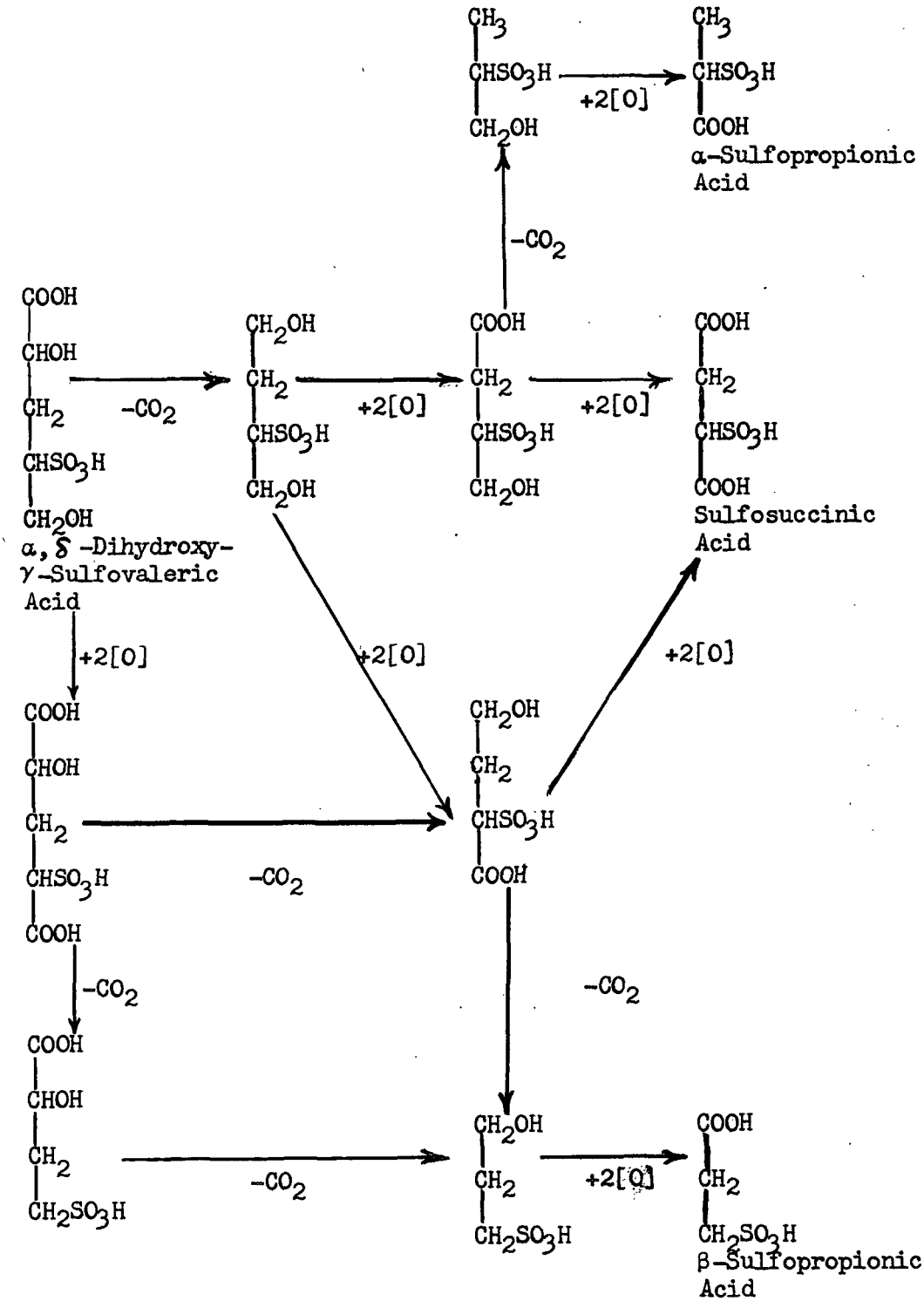


Figure 6. Hypothetical Chromic Acid Oxidation Routes of α, δ-Dihydroxy-γ-Sulfovaleric Acid

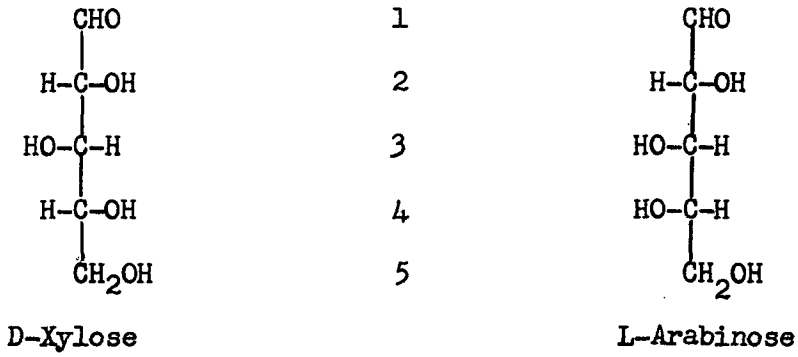
were of value because of the selective reactivity of this oxidant toward 1,2-glycol groupings. The decolorized xylo- and arabo-sulfonic acids were shown to be a mixture of substances consisting of approximately 59% periodate oxidizable material. About 12% of the oxidizable materials or 7% of all the substances in the decolorized sugar-sulfonic acids contained a terminal 1,2-glycol as shown by the quantity of formaldehyde evolved during the oxidation. Another oxidation product was obtained by precipitation of its barium salt in ethanol. Besides the formaldehyde generated, the residual material retained an aldehyde grouping indicating that the original 1,2-glycol must have contained a secondary (rather than a tertiary) alcohol.

An attempt was made to follow the evolution of aldehydes during the periodate oxidations by an alkaline hypoiodite oxidation technique (32). It was noted that the amount of total aldehydes as determined by this technique was very high at the beginning of the periodate oxidations. In order to check the validity of these results, the decolorized sugar-sulfonic acids themselves were subjected to an alkaline hypoiodite oxidation, and the consumption of hypoiodite was found to be 1.5 moles per mole. The nature of this reaction was not easily envisioned since no other evidence of a reducing carbonyl in the decolorized sugar-sulfonic acids was found.

FORMATION OF THE SUGAR-SULFONIC ACIDS

The data which were collected in this thesis have shown that the sugar-sulfonic acids prepared from D-xylose and L-arabinose according to the

procedure of Hägglund and co-workers (6) and Adler (7) were identical substances. On the basis of this fact, several conclusions may be made concerning the formation of the sugar-sulfonic acids. The steric configurations of the two starting sugars are shown in Figure 7. Carbon atoms 2, 3, and 4 are asymmetric centers in both cases, but the configuration of the two sugars differs only at carbon atom number 4. If



D-Xylose L-Arabinose

Figure 7. Structural Formulae of D-Xylose and L-Arabinose

the asymmetry at carbon atoms 2 and 3 were not randomized during the reaction forming the sugar-sulfonic acids, the resulting products, while identical, would be optically active. Similarly, if the asymmetry at carbon atom number 4 were not randomized during the preparative reaction, the products from the two sugars while being stereoisomers would have different optical activities. Since the crude and decolorized sugar-sulfonic acids from both sugars were optically inactive, all the asymmetric centers of the starting sugars must have been completely randomized. Therefore, in any hypothetical mechanism of formation of the five carbon sugar-sulfonic acids, the functional groups on each of these carbon atoms must enter into some type of reaction which results in their randomization.

On the basis of the fact that D-xylose and L-arabinose have been shown to yield identical sulfonic acids together with the above analysis which showed that the asymmetric centers of the two starting sugars had been completely randomized, it would appear that all pentose sugars would yield identical sulfonic acids.

As a result of the investigations of the gluco-sulfonic acids, Adler (7) suggested that the sugar-sulfonic acids may be formed in a manner similar to that of the saccharinic acids. Adler's method of analyzing the gluco-sulfonic acids may be applied to the five carbon sugar-sulfonic acids in the following manner:

The five carbon sugar-sulfonic acids analyzed as $C_4H_8O_2(COOH)(SO_3H)$. If a hydroxyl (OH) replaces the sulfonic acid group, the composition would be $C_4H_9O_3(COOH)$ or $C_5H_{10}O_5$. This composition is the basic formula of the pentose sugars in spite of the fact that the acids contain a carboxyl group. The carboxyl group must have been formed through an intramolecular replacement of oxygen. This type of replacement is characteristic of the formation of the saccharinic acids. Adler stated, "it seems probable that the same type of reactions (formation of the saccharinic acids) may account for the formation of our sulfosaccharinic acid."

Adler suggested a means by which the gluco-sulfonic acids were formed. This scheme was "represented to give an idea of how the formation of an acid of the B-acid type (one of two gluco-sulfonic acids investigated) may take place. This scheme does not in any way claim to give the real reaction, but only shows one of many hypotheses."

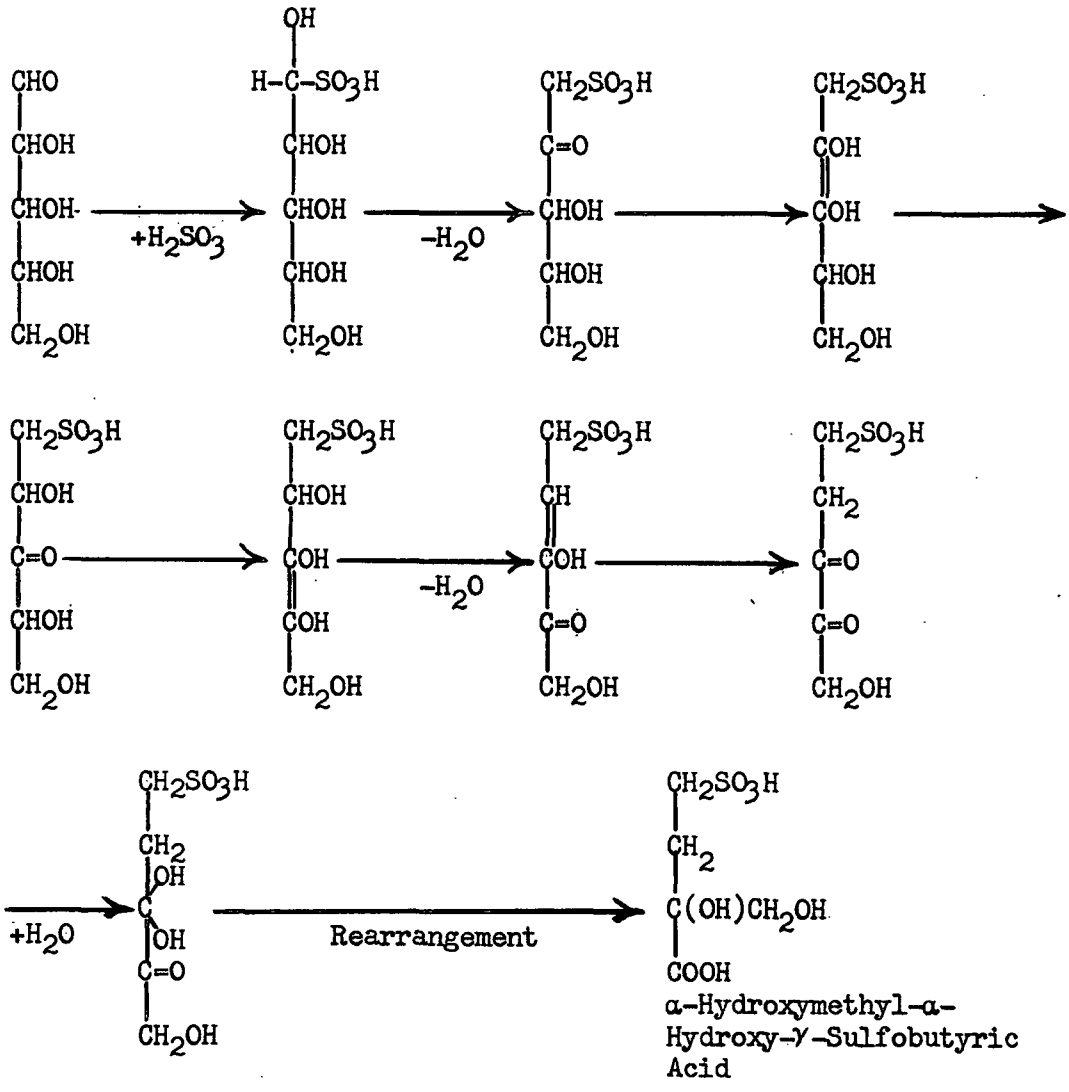
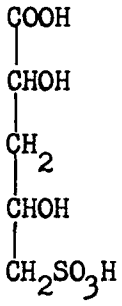


Figure 8. Mechanism of Formation of α-Hydroxymethyl-α-Hydroxy-γ-Sulfobutyric Acid (7)

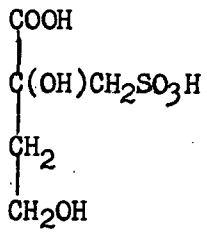
Adler's scheme modified to five carbon acids is shown in Figure 8. The same qualification as applied by Adler to his scheme applies in this case also. In this hypothetical reaction mechanism, the first step involves the formation of an aldehyde-bisulfite addition complex. This compound then undergoes an intramolecular oxido-reduction so that the

sulfonic acid group is stabilized by being attached to a methylene group, and a ketone is formed at carbon atom number 2. The newly formed carbonyl function progresses through the whole chain by a continued change between H and OH groups. This same type of reaction has been postulated in the formation of the saccharinic acids. As the final step, a "benzylic acid rearrangement", which has been postulated in the formation of the saccharinic acids, takes place to yield an α-hydroxymethyl-α-hydroxy-γ-sulfobutyric acid.

Variations of this scheme can also be postulated to yield α, γ-dihydroxy-δ-sulfovaleric and α-sulfomethyl-α,γ-dihydroxybutyric acids shown in Figure 9.



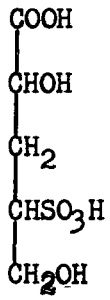
α,γ-Dihydroxy-δ-Sulfovaleric Acid



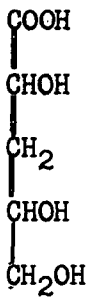
α-Sulfomethyl-α,γ-Dihydroxy Butyric Acid

Figure 9. Structural Formulae of α,γ-Dihydroxy-δ-Sulfovaleric and α-Sulfomethyl-α,γ-Dihydroxy Butyric Acids

Adler's hypothesis that the sugar-sulfonic acids may be formed by a method analogous to that of the saccharinic acids is strengthened by comparing the structure of the pure xylo-sulfonic acid shown by Yllner (8) to be α,δ-dihydroxy-γ-sulfovaleric acid with the five carbon metasaccharinic acids as shown in Figure 10.



α,δ-Dihydroxy-γ-Sulfovaleric Acid



Metasaccharinic Acid

Figure 10. Xylo-Sulfonic Acid (8) and Metasaccharinic Acid

Although the relationship between the "A" sugar-sulfonic acids isolated in this thesis to Yllner's pure xylo-sulfonic acid was not definitely shown, the data indicated that the acids were identical substances.

Adler's proposed mechanism of formation (2) does not directly account for the formation of α,δ-dihydroxy-γ-sulfovaleric acid. However, using his basic hypothesis that the reaction is similar to the formation of the saccharinic acids, it is possible to construct a series of hypothetical reactions with α,δ-dihydroxy-γ-sulfovaleric acid as the final product. In this case, the reaction mechanism is similar to the hypothetical mechanism to form a five carbon metasaccharinic acid shown in Figure 11 (36). The hypothetical mechanism of formation of α, δ-dihydroxy-γ-sulfovaleric acid is shown in Figure 12.

Considering the various steps in the hypothetical reaction route shown in Figure 12, the following steps are based largely upon the hypothesis that the route is similar to that of the metasaccharinic acids:

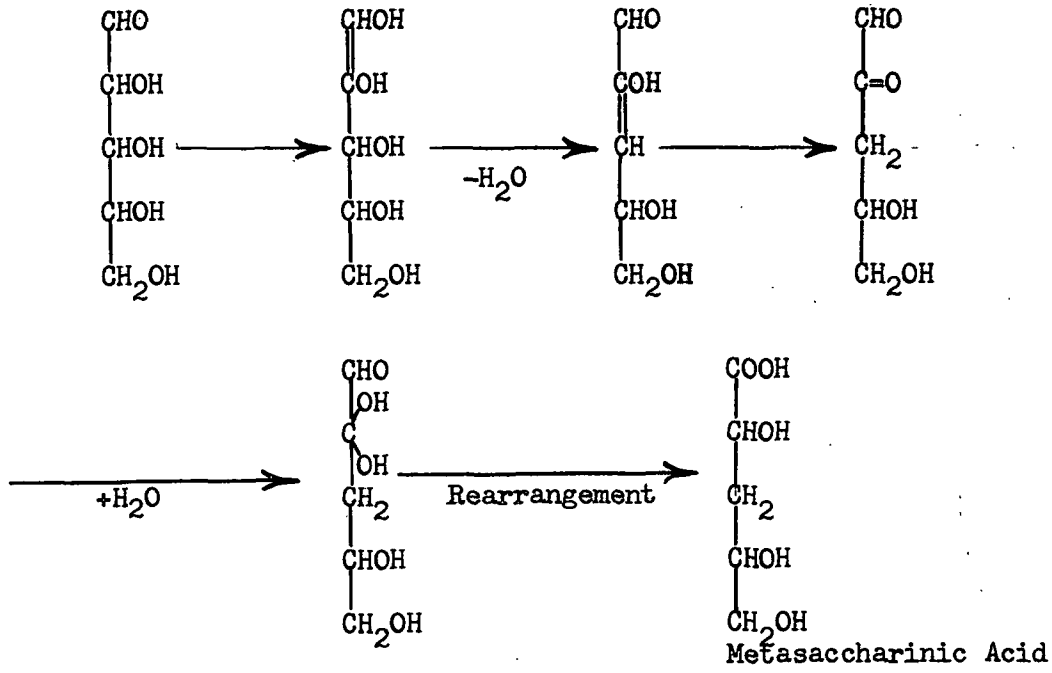


Figure 11. Mechanism of Formation of Metasaccharinic Acid (36)

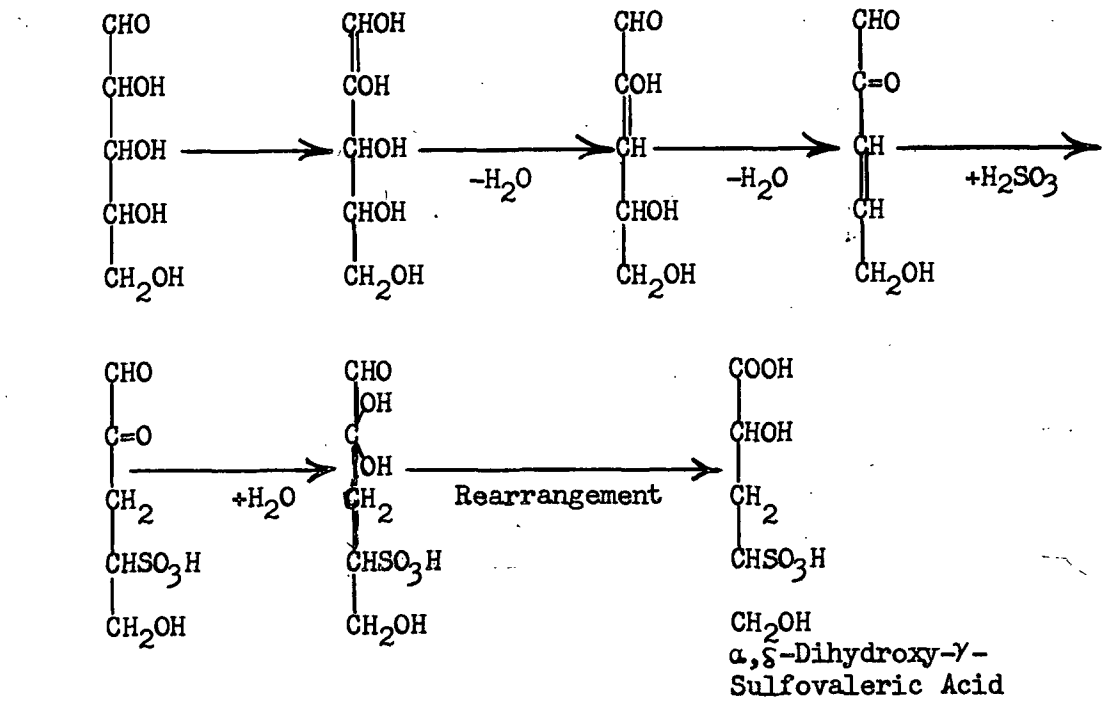
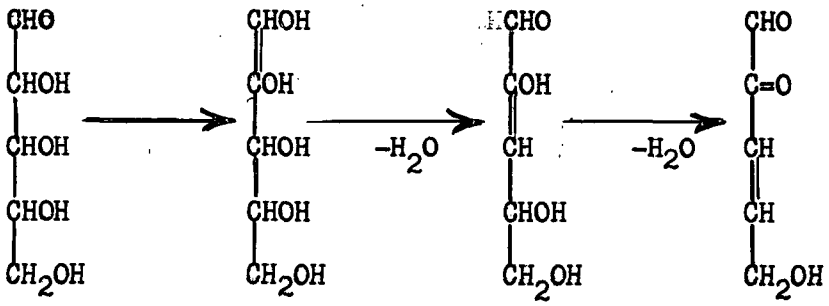
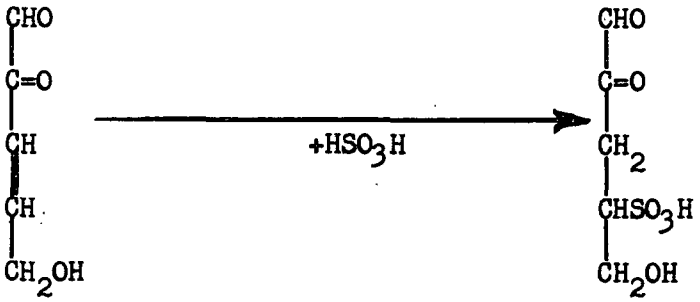


Figure 12. Mechanism of Formation of α,δ-Dihydroxy-γ-Sulfovaleric Acid

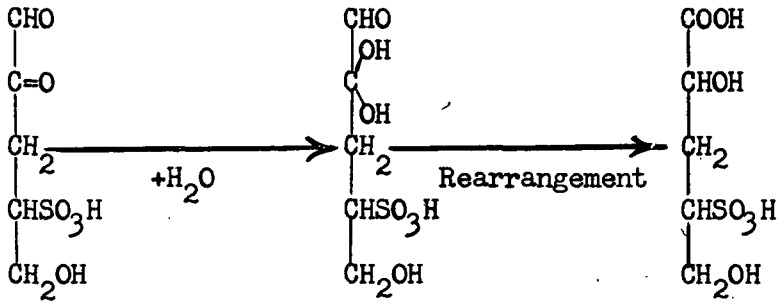


A considerable number of sulfonic acids have been prepared by the addition of an alkali bisulfite to an unsaturated ketone or aldehyde in which the double bond is conjugated with the carbonyl group (37). Thus, sulfonation may take place as follows:



There is strong evidence that the sulfonic acid group would enter β to the keto-carbonyl group (8, 31, 38, 39). The resulting sulfonated product could then add water and undergo a rearrangement as has been postulated in the formation of the saccharinic acids (36).

The mechanisms which have been presented do not necessarily represent the true reactions involved in the formation of the sugar-sulfonic acids. Similarly, the products do not necessarily represent the nature of the products actually composing the sugar-sulfonic acids. The discussion is purely hypothetical and may, therefore, be useful in understanding these very complex substances, but is based on only one of many possible hypotheses.



α,δ-Dihydroxy-γ-
Sulfovaleric Acid

CONCLUSIONS

The sulfonic acids derived from D-xylose and L-arabinose have been investigated. The acids were prepared from the pentose sugars by treatment with hot, nearly neutral (pH 6.3-6.6) sodium sulfite-bisulfite solutions according to the general procedure of Hägglund and co-workers (6) and Adler (7). The yield of the crude acids, as the barium salts, was approximately 47% based on the initial sugar. The fate of the other 53% of the initial sugar was not investigated. The following conclusions may be made concerning the nature of the xylo- and arabo-sulfonic acids studied in this thesis.

1. During the reaction producing the crude xylo- and arabo-sulfonic acids, the asymmetric centers of the starting sugars were randomized as shown by the optical inactivity of the crude acids.

2. The crude xylo- and arabo-sulfonic acids were shown to be sulfocarboxylic acids by elemental analysis of their barium salts. The barium-to-sulfur ratios were 0.92 and 0.94 for the xylo- and arabo-sulfonates, respectively. A pure barium sulfocarboxylic acid salt theoretically would have a barium-to-sulfur ratio of 1.00.

3. Since the chromatographic properties of the crude xylo- and arabo-sulfonic acids were also identical, the acids probably were identical materials.

4. The crude xylo- and arabo-sulfonic acids were shown to be mixtures of at least six components since, when chromatographed on Whatman 3MM paper using EAm developer, six acidic spots, termed 1', 2', 3'a, 3'b, 3'c, and 4' were found.

5. The crude xylo- and arabo-sulfonic acids were at least partially purified by treatment with animal charcoal-celite mixtures since the resulting products were chromatographically homogeneous (corresponding to spot 2 or 2' of the crude acid mixtures) in PEAm and EAm developers.

6. The decolorized barium xylo- and arabo-sulfonates analyzed as $C_5H_8O_7SBa.H_2O$. Since the barium-to-sulfur ratios were 0.97 and 0.99 for the xylo- and arabo-sulfonates, respectively, the acids were sulfocarboxylic acids.

7. The decolorized xylo- and arabo-sulfonic acids did not contain aldehyde or ketone groups as shown by their inactivity toward phenylhydrazine and Tollens reagent (24). As a result, the oxygen not found in the sulfonic and carboxylic acid functions was tentatively assigned to two hydroxyl groups.

8. Since both the decolorized xylo- and arabo-sulfonic acids were optically inactive, they probably were composed of d,l pairs of stereoisomers.

9. The infrared spectra of the decolorized barium xylo- and arabo-sulfonates were identical showing that the acids themselves were identical materials. Since the xylo- and arabo-sulfonic acids were optically inactive indicating that the asymmetric centers of the starting sugars had been randomized, theoretically, all pentose sugars should yield identical products. Although no studies of the mechanism of formation of the sugar-sulfonic acids were made in this thesis, it seems possible that the mechanism is similar to that of the saccharinic acids (7).

10. During the course of chromic acid oxidations of the decolorized

xylo- and arabo-sulfonic acids, approximately 4 atoms of oxygen were consumed and 1.67 moles of carbon dioxide were evolved per $C_5H_{10}O_7S$ unit. In addition, sulfosuccinic, and α- and β-sulfopropionic acids were shown to be oxidation products. These data were not useful in diagnosing the nature of the decolorized xylo- and arabo-sulfonic acids except to further substantiate the hypothesis that the two sugar-sulfonic acids were identical materials.

11. The decolorized xylo- and arabo-sulfonic acids were shown to be mixtures of at least three types of compounds by periodic acid oxidation. These types may be classified as follows:

a. Approximately 41% of the decolorized xylo- and arabo-sulfonic acids consisted of materials which did not contain any 1,2-glycol groups as shown by their stability toward periodate.

b. Approximately 7% of the decolorized xylo- and arabo-sulfonic acids consisted of materials containing terminal 1,2-glycol groups as shown by the quantity of formaldehyde evolved during the reaction.

c. Approximately 52% of the decolorized xylo- and arabo-sulfonic acids consisted of material containing 1,2-glycol groups which were nonterminal.

Another periodate oxidation product besides the formaldehyde was shown to be an acidic aldehyde by its reactions with 2,4-dinitrophenylhydrazine and Tollens reagent (24). Thus, the original 1,2-glycol must have contained a secondary rather than a tertiary alcohol.

12. The chemical analyses, ultraviolet and infrared spectra, and specific rotations of the crystalline brucine salts of the "A" sugar-sulfonic acids

being essentially identical further substantiated the conclusion that the xylo- and arabo-sulfonic acids were identical substances. Furthermore, the methoxyl and brucine-to-sulfur ratios being near the theoretical values of 4 (4.09 and 4.05) and 2 (2.02 and 2.01), respectively, indicated that the "A" acids were sulfocarboxylic acids.

13. Similarly, the chemical analyses, infrared spectra, and optical activities of the crystalline barium salts of the "A" sugar-sulfonic acids being essentially identical further substantiated the conclusion that the xylo- and arabo-sulfonic acids were identical substances. Again, the barium-to-sulfur ratios being near the theoretical value of 1.00 (1.06 and 1.04) indicated that the "A" acids were sulfocarboxylic acids.

14. Since the "A" xylo- and arabo-sulfonic acids were optically inactive, they probably were composed of d,l pairs of stereoisomers.

15. The "A" acids did not contain any 1,2-glycol groups since they were stable toward periodate.

16. Although the relationship was not definitely established, the "A" acids isolated in this thesis had many properties in common with the pure xylo-sulfonic acid shown by Yllner (8) to have the structure of an α,δ-dihydroxy-γ-sulfovaleric acid. These similar properties may be listed for comparison as follows:

Brucine salts: elemental analysis as $C_5H_{10}O_7S.2C_{23}H_{26}O_4N_2$; crystalline form as short rods.

Barium salts: elemental analysis as $C_5H_8O_7S\ Ba.H_2O$; crystalline form as rods; specific rotation = 0°; stability toward periodate.

17. The barium salts of the "B" fraction had been at least partially resolved as shown by their optical activity. This fraction was a mixture of substances as indicated by the fact that it was oxidized by periodate in less than a stoichiometric amount (0.307 mole periodate consumed per $C_5H_{10}O_7S$ unit). The fact that the periodate consumption was less than that found for the decolorized sugar-sulfonic acids appears to indicate that the "B" acids were enriched with the periodate-stable "A" acids. The noncrystallinity of the "B" and decolorized barium sugar-sulfonates may have masked their infrared spectra to such a degree that they appeared almost identical.

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APPENDIX I

ANALYSIS FOR CARBON DIOXIDE DURING CHROMIC ACID OXIDATIONS

The quantity of carbon dioxide evolved during the oxidations was measured as follows: The oxidizing medium, consisting of 0.5 g. of potassium dichromate and 4.4 g. of concentrated sulfuric acid in a 40-ml. aqueous solution, was placed in a uronic acid apparatus (40). The liquor was heated to 100°C. and swept with carbon dioxide-free nitrogen. The gases were passed through a cold water condenser, a concentrated sulfuric acid bubbler, and a magnesium perchlorate column. Carbon dioxide was then absorbed in a tared Ascarite[1]-magnesium perchlorate tube. After the absorption tube had attained a constant weight, the liquor was cooled to room temperature with the flow of nitrogen maintained through the apparatus. The decolorized xylo- or arabo-sulfonic acid (0.0975 g.) in 10 ml. of solution was then introduced into the reaction flask and the oxidation was run at 100°C. for the required time. Upon completion of the reaction, the tared absorption tube was reweighed to determine the quantity of carbon dioxide evolved.

[1] A product of the Arthur H. Thomas Company.

APPENDIX II

SYNTHESIS OF BARIUM SULFOSUCCINATE

Sulfosuccinic acid was prepared from fumaric acid by the method of Backer and van der Zanden (28). An amount of 11.6 g. of fumaric acid was neutralized to the phenolphthalein end point with 10N potassium hydroxide. The resulting solution was heated to about 60°C., and 12.2 g. of potassium metabisulfite were added. The reaction liquor was then boiled for 8 hours. After cooling, the liquor was diluted with water, and 10 ml. of concentrated hydrochloric acid were added. The sulfur dioxide which evolved was swept away by a current of air. A small amount of barium chloride was then added to remove the sulfate ions which were formed during the reaction. The solution was filtered and neutralized with potassium hydroxide. It was then heated to 100°C., and 32 g. of barium chloride were added. The barium sulfosuccinate precipitated immediately and, after being washed with hot water until the washings were chloride free, was obtained in a yield of 34.5 g. or 86.4% of the theoretical. (Found: Ba 50.5, S 8.09, C 11.6, H 1.22 - Calculated for $C_4H_3O_7SBa_{1.5}$. Ba 51.3, S 8.00, C 12.0, H 0.75). The infrared spectrum of this material is shown in Figure 13.

APPENDIX III

ISOLATION OF SULFOSUCCINIC ACID FROM CHROMIC ACID OXIDATION LIQUORS

Upon completion of the oxidation, formaldehyde was added to the hot (100°C.) liquor to destroy the excess oxidant. After cooling, the liquor was neutralized with ammonium hydroxide, and the resulting insoluble chromic hydroxide was removed by filtration[1]. The filtrate was passed through a column of Amberlite IR-120 in the acid form to convert the ammonium salts to the free acids. After neutralizing with sodium hydroxide, the liquor was taken to dryness and extracted with 80% ethanol for 24 hours. The extract, termed "crude sulfopropionic acid fraction," is discussed in Appendix IV. The residue was treated with hydrochloric acid equivalent to the maximum theoretical yield of sulfosuccinic acid. The resulting solution was concentrated at 50°C. under reduced pressure, and 95% ethanol was added periodically until only a small amount of solids was precipitated upon addition of more ethanol. The insoluble salts were removed by filtration, and the ethanol was stripped from the filtrate. The resulting liquor was neutralized with ammonium hydroxide and taken to dryness. It was reacidified with hydrochloric acid, and the precipitation process with ethanol was repeated. The resulting acidic liquor was treated with barium chloride to precipitate the remaining sulfate ion. After removing the barium sulfate, the liquor was concentrated at 50°C. under reduced pressure to a small volume. It was then

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[1] Attempts to remove chromic ions on columns of Amberlite IR-120 in the acid form were unsuccessful because of the amphoteric nature of this ion. These findings were in accord with the data of Samuelson (41).

neutralized with potassium hydroxide to the phenolphthalein end point and heated to near the boiling point. Upon adding barium chloride, the water-insoluble barium salt of sulfosuccinic acid precipitated and was centrifuged off. The salt was washed with water until the washings were neutral and chloride free. It was then washed with absolute ethanol and ether and dried at 60°C. under reduced pressure over phosphorus pentoxide. When this isolation procedure was applied to facsimiles of the oxidation liquors containing known amounts of sulfosuccinic acid, a recovery of approximately 50% was effected.

Application of this recovery procedure to oxidation liquors of the decolorized xylo- and arabo-sulfonic acids resulted in the isolation of barium sulfosuccinate in yields of 12.3 and 14.7%, respectively, based on total conversion of the sugar-sulfonic acids to sulfosuccinic acid. The infrared spectra of these samples were identical to that of the authentic material as shown in Figure 13.

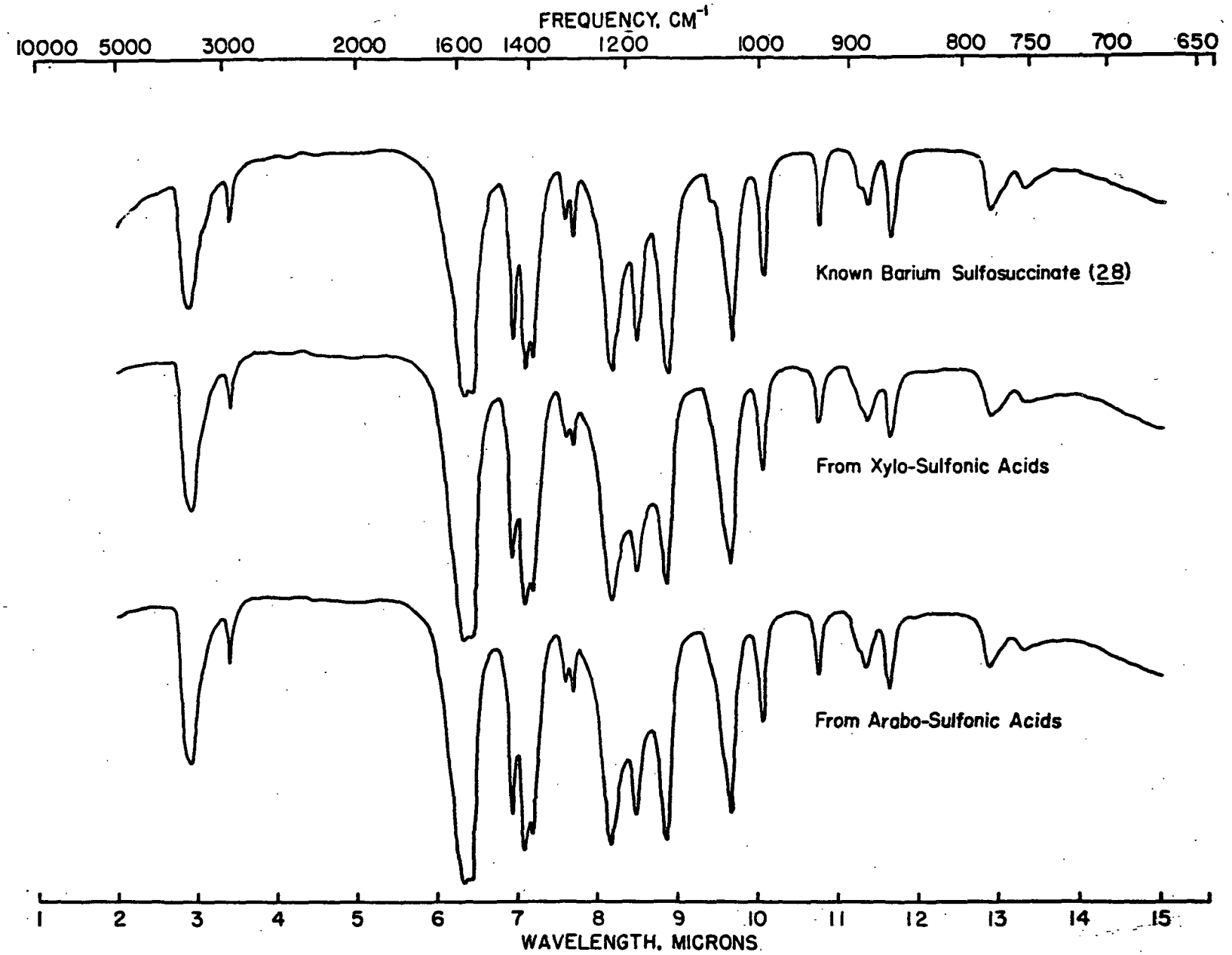


Figure 13. Infrared Spectra of Barium Sulfosuccinate

APPENDIX IV

ISOLATION OF ALPHA- AND BETA-SULFOPROPIONIC ACIDS FROM THE CHROMIC ACID OXIDATION LIQUORS

The alcoholic solution of the "crude sulfopropionic acid fraction" was heated under reduced pressure to strip off most of the ethanol. The resulting aqueous solution was passed through a column of Amberlite IR-120 in the acid form to convert the sodium salts into the free acids. After heating to near the boiling temperature, the acids were neutralized with barium hydroxide and filtered while hot. The filtrate was concentrated to a small volume at 50°C. under reduced pressure and poured into two volumes of 95% ethanol to precipitate the barium salts. After standing several hours to assure complete precipitation, the salts were removed by filtration, lightly washed with water, absolute ethanol and ether, and dried at 60°C. under reduced pressure over phosphorus pentoxide. The yields of the impure materials were 11.1 and 10.3% based upon total conversion of the decolorized xylo- and arabo-sulfonic acids, respectively, to sulfopropionic acid. (Found: From xylo-sulfonic acid Ba 48.1, equivalent weight 147.5; From arabo-sulfonic acid Ba 48.1, equivalent weight 146.2 — Calculated for $C_3H_4O_5SBa$: Ba 47.5, equivalent weight 144.5).

Chromatographic studies of the crude free acids using developer EAm indicated that at least four acid components were present. These four components were separated on Whatman 3MM paper sheets 7 inches in width using the EAm developer. Fifty milligrams of the crude free acids were

applied to each sheet; the total quantity applied being 50 and 200 mg. of the crude products derived from the xylo- and arabo-sulfonic acids, respectively.

After a development time of 48 hours, the chromatograms were air dried, and guide strips were cut from the center of each sheet. The acid components were located on these strips by the formaldehyde-methyl orange spray technique (see pages 21 and 22). The four acid components, A, B, C, and D, had moved 0, 3, 12, and 33 cm., respectively, from the origin.

The components were eluted from the paper sheets with boiling water and passed through a column of Amberlite IR-120 in the acid form to convert the ammonium salts into the free acids. After an initial concentration, the acids were neutralized with barium hydroxide to form the barium salts. The solutions were then treated with a small amount of activated carbon to remove colored impurities and concentrated to a small volume at 50°C. under reduced pressure. The barium salts were precipitated by pouring the concentrated solutions into 95% ethanol. The total recovery of chromatographed material derived from xylo- and arabo-sulfonic acids was 48.3 and 43.8%, respectively.

Components A and B were not identified. Component C was shown to be barium α-sulfopropionate by comparing its infrared spectrum with that of the authentic material which was synthesized according to the method of Backer and Dubsky (29) (see Appendix V). Similarly, component D was identified as barium β-sulfopropionate by comparing its infrared spectrum

with that of the authentic material which was synthesized by the procedure of Kharasch, Chao, and Brown (30) (see Appendix VI). The spectra of the α-sulfopropionic acids are shown in Figure 14, and those of the β-sulfopropionic acids are given in Figure 15. In addition, the S-benzyl-iso-thiourea salt of component D was prepared (31) and found to melt at 151 to 152°C.[1] Its melting point was not depressed when mixed with the salt of the authentic acid. The yields of the various components are given in Table IV.

TABLE IV

YIELDS OF THE FOUR COMPONENTS SEPARATED BY CHROMATOGRAPHY FROM THE CRUDE SULFOPROPIONIC ACID FRACTION

Component	Yield, %[a] Derived From Xylo-Acids	Arabo-Acids
A, Unknown	2.4	1.2
B, Unknown	2.8	2.8
C, α-Sulfopropionic acid	2.9	2.6
D, β-Sulfopropionic acid	3.1	3.7

[a] Corrected for losses in chromatographing, and based on total conversion of the sugar-sulfonic acids into sulfopropionic acid.

[1] Uncorrected

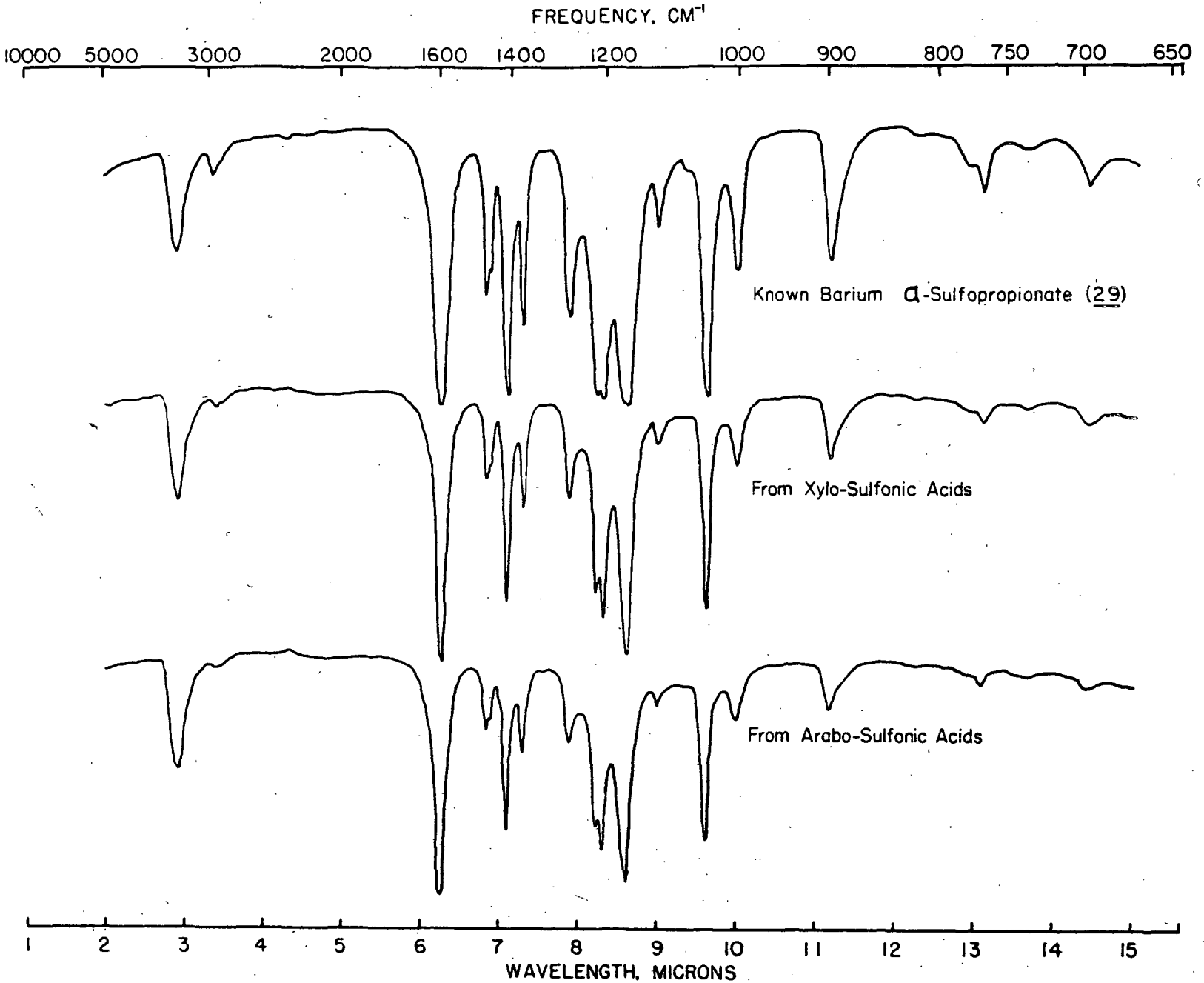
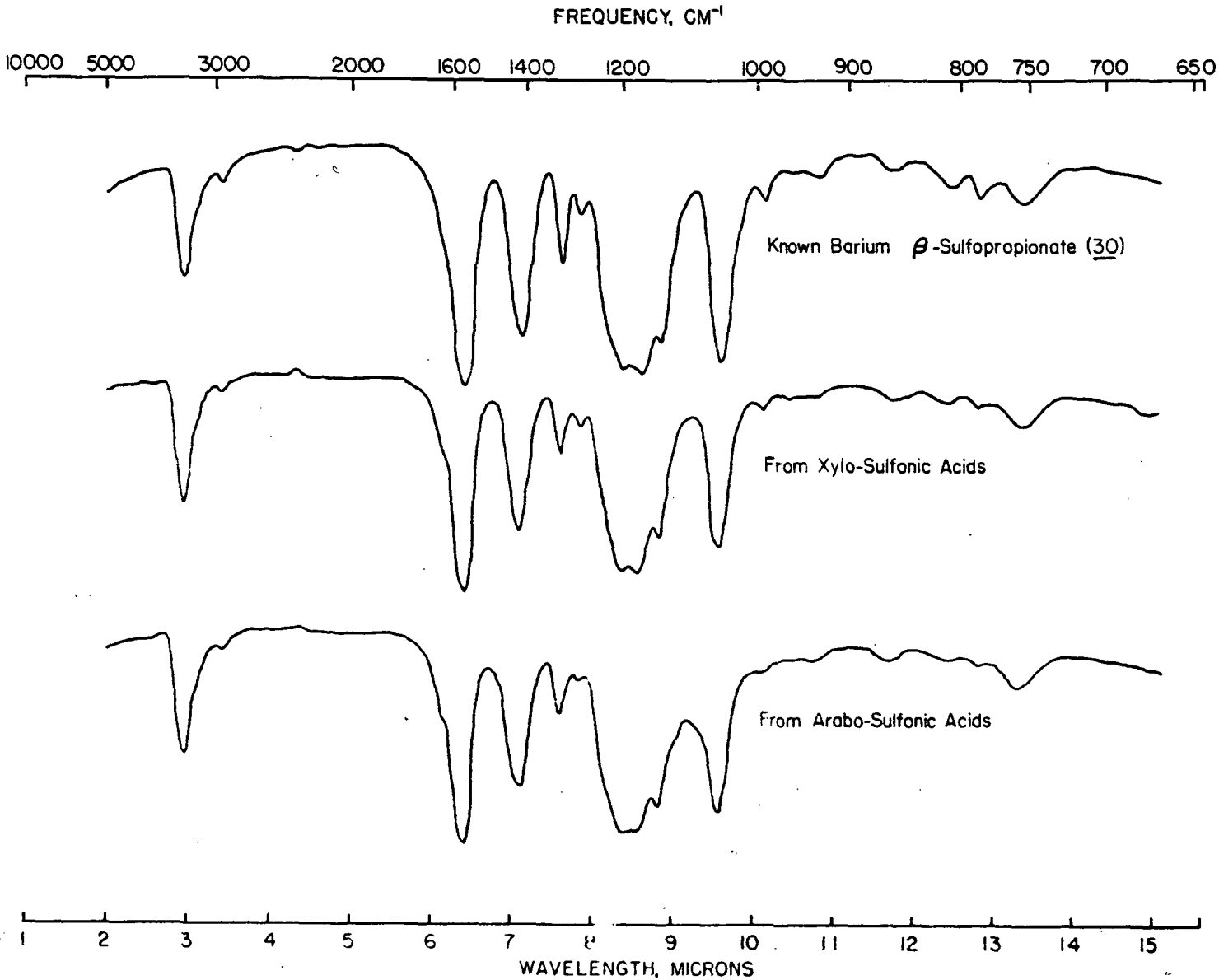


Figure 14. Infrared Spectra of Barium α-Sulfopropionate



Fibure 15. Infrared Spectra of Barium β-Sulfopropionate

APPENDIX V

SYNTHESIS OF BARIUM α-SULFOPROPIONATE

Barium α-sulfopropionate was synthesized by the method of Backer and Dubsky (29). A volume of 14 ml. of fuming sulfuric acid (assay 30 to 33% free SO_3) was added slowly and with thorough mixing to 13 grams of propionic anhydride. The reaction was maintained cool in an ice-salt bath. A colorless sirup formed.

On removing the mixture from the ice bath, considerable heat was given off, and the material developed a brown color. Caution was taken to prevent the temperature from rising too rapidly. The reaction was completed by slowly raising the temperature to 90°C. and maintaining the mixture at that temperature for 1/2 hour.

After cooling, the reaction mixture was diluted to between 100 and 200 ml. with water. The unreacted propionic acid was then removed by steam distillation. The residue, after diluting to 1000 ml. with water, was neutralized with excess barium carbonate and filtered. The filtrate was concentrated on a steam bath, and precipitated barium α-sulfopropionate was recovered by filtration. The liquid was not taken to dryness as the final product was highly colored and difficult to purify (29).

The barium salt was purified as follows: The salt was dissolved in 16 times its weight of hot water, filtered if necessary, cooled, and precipitated in twice the volume of 95% ethanol. This purification process

was repeated twice. (Found: Ba 47.2, S 11.0, equivalent weight 149 — Calculated for $C_3H_4O_5SBa$: Ba 47.5, S 11.1, equivalent weight 144.5). The infrared spectrum of this material is shown in Figure 14 (see Appendix IV).

APPENDIX VI

SYNTHESIS OF BARIUM β-SULFOPROPIONATE

Barium β-sulfopropionate was prepared by the method of Kharasch, Chao, and Brown (30). An amount of 74 grams of anhydrous propionic acid and 185.6 grams of sulfuryl chloride were placed in a round-bottomed glass flask which was then attached to an efficient reflux condenser. The flask was illuminated by a tungsten filament lamp (300 watts) placed about 5 cm. distant. The reaction was run at 55°C. overnight.

Dissolved gaseous products and excess sulfuryl chloride were removed under reduced pressure from the crude β-sulfopropionic anhydride. Approximately 200 ml. of ligroin-benzene (80:20) were added to complete the separation of solids and to facilitate filtration. After filtering, the precipitate was dried in a vacuum desiccator containing freshly fused sodium hydroxide, phosphorus pentoxide, and paraffin.

The resulting product, which still smelled strongly of sulfuryl chloride, weighed 58 grams corresponding to a yield of 42.7%. The crude product was dissolved in 1750 ml. of water and neutralized with potassium hydroxide. The solution was heated to boiling, and 93 grams of barium chloride dihydrate were added. The solution was filtered while still hot to remove the barium sulfate which formed. Upon cooling, the barium β-sulfopropionate crystallized in the form of thin plates. The crystals were removed by filtration, and the filtrate was concentrated on a steam bath to a volume of 600 ml. Upon cooling, a second crop of crystals was obtained. The barium β-sulfopropionate was recrystallized

once. The crystals were washed with water and dried at 60°C. under reduced pressure over phosphorus pentoxide. The yield was 108 grams or 37.4% of the theoretical. (Found: Ba 46.6, S 11.1, equivalent weight 150 -- Calculated for $C_3H_4O_5SBa$: Ba 47.5, S 11.1, equivalent weight 144.5). The infrared spectrum of this material is shown in Figure 15 (see Appendix IV).